***Salvia divinorum**:*

The Botany, Ethnobotany, Biochemistry and Future of a Mexican Mint

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Abstract

Salvia divinorum (Labiatae) is an entheogen used by the Mazatec Indians of the Sierra Mazateca in Oaxaca, Mexico. *S. divinorum* was introduced to the scientific community in the 1950's, and has since become the subject of ethnobotanical, botanical, and biochemical research. Plant biologists are interested in *S. divinorum* due to its anthropogenic distribution and limited sexual reproduction, while biochemists have found that *S. divinorum* contains one of the most potent natural hallucinogens known: salvinorin A. Ethnobotanically, the Mazatec shamans used the plant for healing, divination, and shamanic training, and the spiritual qualities of *S. divinorum* may now contribute to its growing popularity among the general public, as experimental users seeking to "expand consciousness" order *S. divinorum* over the internet. The many applications and mysteries of *Salvia divinorum* have led to numerous research opportunities, and the plant may become more important both pharmacologically and socially worldwide.

Botany of *Salvia divinorum*

Salvia divinorum is a perennial herb in the Labiatae, which grows in the highlands of the Sierra Mazateca, Oaxaca, Mexico. One of almost 1,000 species of *Salvia* in the world, *S. divinorum* is recently discovered by western science, but has become rapidly well known for its hallucinogenic properties, but has also been researched for its unusual botanical characteristics.. Traditionally, the herb has been used in healing and divination among the Matazec of Oaxaca.

The first introduction to *S. divinorum* came in 1938 when Jean B. Johnson, son-in-law of anthropologist Roberto Weitlaner, wrote about *Hierba Maria* in his publications about the Mazatec shamans (1939). In 1945, Blas P. Reko also found the magical plant among the Cuicatecs and Mazatecs which produced visions, referred to as "leaves of prophecy," but could not identify the plant from the loose leaves collected. Weitlaner also conducted interviews

concerning the magical plant, *Yerba de Maria*, but no identification was made until 1957, when Arturo Gomez-Pompa collected the plant while collecting mushrooms for the drug firm CIBA. He obtained enough of the plant, called *xka Pastora*, to identify it as a *Salvia* sp; however, no flowering material was available, and Gomez-Pompa could not identify the plant further (Valdes 2001a). Finally, in 1962, Gordon Wasson and Albert Hoffman undertook the goal to find and definitively research the purported "magical" plant. Hoffman, who had already discovered LSD and isolated psilopsybin and lysergic acid amides from mushrooms and morning glory seeds used by the Mazatec, joined with the leadership of self-styled ethnomycologist Gordon Wasson to traverse the Sierra Mazateca looking for *S. divinorum,* particularly in the wild. Wasson and Hoffman could not find independent populations of *S. divinorum*, but obtained the first flowering specimens of the plant from an old *curandera*, Natividad Rosa, in the village of San Jose Tenango. Wasson and Hoffman were not allowed to visit the location where the plants grew, leading Wasson to conclude that *S. divinorum* was probably a cultigen (Wasson 1962). The specimens were identified as a new species of Salvia by Carl Epling and Carlos D. Jativa at the Botanical Institute of Harvard University in Cambridge, Massachusetts (Epling and Jativa 1962).

S. divinorum is described as a perennial herb, mostly 1-1.5 m tall, with taller stems often decumbent, enabling plant to reproduce vegetatively by rooting at nodes and sometimes internodes, and to resprout vigorously from dry, senescent stem material. It has several characteristics related to other *Salvia* sp., including a quadrangular stem, serrated leaves situated oppositely on the stem, and sigmoidal flowers growing on tall racemes(Epling and Jativa 1962). However, it is highly distinctive and unusual among the *Salvia* species is certainly anomalous in sect. *Dusenostachys* Epl., to which it was originally assigned (Epling & Jativa 1962, Reisfield 1993).. According to Epling and Jativa, *S. divinorum* may be allied to *S. cyanea* Lamb ex. Benth, which is also found in central Mexico, but differs in the leaf shape and the flattened upper style branch (Epling and Jativa 1962). In the original description of *S. divinorum,* Epling (1962) described the plant as having a blue calyx tube and corolla, an error which persisted in some literature, including the first edition of Emboden's book *Narcotic Plants* and R.E. Shultes' *Hallucinogenic Plants*, among others. Emboden was the first to correct this error, however, in his

second edition of *Narcotic Plants* when he described *S. divinorum*'s corollas as "pure white" (Ott 1996). The official description of *S. divinorum* was amended again by Reisfield in 1993, who described the reproductive parts of the plant in detail, including the nutlets, which are about 2 mm long and dark brown when viable.

Distribution is limited to the highlands of Sierra Mazateca, where it grows at elevations of 300-1800 m in primary and secondary cloud forest or tropical evergreen forest. *S. divinorum* prefers to grow in the black soils along streambanks, where it spreads vegetatively in heavily shaded, moist ravines. *S. divinorum* has also been found planted in coffee plantations, which are frequently blanketed in heavy fog, providing necessary humidity to grow. *S. divinorum* is usually anthropogenically distributed, grown in cultivated or semi-cultivated populations that are well hidden by the Mazatec, (Wasson 1962, Valdes 1983, 1987, Reisfield 1993). Because of the Mazatec's secrecy, ethnobotanists were unable to identify the plant for many years because they were not allowed to visit the growing sites, and flowering material was required for a definitive identification (Wasson 1962, Hoffman 1990).

Since 1962, the botanical characteristics of *S. divinorum* have been studied in greater detail, particularly regarding its flowering and seed set. (Valdes 1982, 1983, 1987, Reisfield 1993). By mapping the known Mexican populations of *s. divinorum*, Reisfield witnessed a few populations in flower, and discovered that while vegetative growth is promoted by cool, wet, shady environments, flowering is promoted by sunlight and may occur anytime from October to May in Mexican populations, though flowering occurs sporadically and infrequently. In Mazatecan populations, seed set has not been observed and all plants appear to be clonal (Reisfield 1993, Valdes 1987). The nectar and dimensions of the *S. divinorum* flower suggest ornithophily, but the only pollination event observed in the wild involved a single hummingbird. It is believed, then, that pollination is opportunistic, rather than the result of a specialized plant-pollinator coevolution (Reisfield 1993). As a result, it is possible that many populations of *S. divinorum* are clonal, but this has not yet been investigated. Valdes asserted in 1987 that all *S. divinorum* in the United States at that time was cloned from the original specimens given to Epling, which was propagated at the University of California, Berkely.

Valdes and Reisfield investigated the reproductive qualities of *S. divinorum* using both collections from the forests of the Sierra Mazateca and clones from the original plants introduced by Hoffman and Wasson. In Valdes' greenhouse experiments, newly collected plants from the field were crossed with the original clones. Of the 14 flowers cross-pollinated, 4 set seed (28%). Valdes was unable to test the viability of these seeds, however (Valdes 1987). Reisfield performed both self-pollination studies and cross-pollination studies. Of the 432 potential seeds that could be formed from the 108 self-pollinations, only 11 nutlets developed (2.5%). Of the 190 cross-pollinated flowers, 24 nutlets developed (3%). Reisfield planted several mature seeds in the greenhouse, and "vigorous seedlings developed which were undistinguishable (though not grown to flowering) from their parents." Reisfield further investigated the reproductivity of *S. divinorum* by experimentally observing that only 53% - 56% of pollen is viable, and adhesion to the stigma is poor. 33% of pollinated styles showed pollen germination and pollen tube growth, and pollen tubes appeared healthy, suggesting that the primary barrier to fertility is not inhibition of the pollen tube. Reisfield suggested that the probably cause of infertility in *S. divinorum*, then, is post-zygotic embryonic abortion due to either inbreeding, hybridity, or a delayed self-incompatibility reaction. The abnormalities of *S. divinorum* seem most closely aligned to characteristics of hybridity; however, no two *Salvia* species have been found that show an obvious affinity to *S. divinorum* (Reisfield 1993). *S. cyanea* may be one potential progenitor (Epling and Jativa 1962), but this has not been tested, and no other *Salvia* species appear to be likely candidates. Reisfield concludes that *S. divinorum* may be a hybrid or an inbred cultigen, but asserts that the origin of *S. divinorum* is still a mystery (1993).

Ethnobotany of S. divinorum:

Though little has been known about *S. divinorum* until recently, it was originally researched primarily because of its fascinating role in the ethnopharmocology and rituals of the Mazatec Indians. *S. divinorum* is one of a suite of local hallucinogens employed for curing, divination, and shamanic training by the Mazatec, and a few other nearby indigenous groups. Ethnobotanists learned of its existence while researching the better-known Mazatecan

hallucinogens: mushrooms and morning glory seeds, but soon took an interest in the “magic leaves” of *S. divinorum.*

S. divinorum is a sacred plant in the Sierra Mazateca, where it is identified with the Virgin Mary, mother of Christ. The common names of *S. divinorum* reflec this relationship; in Mazatec, the plant is known as *ska Maria*, or *ska Pastora,* or in Spanish, *Hojas de la Pastora, Hojas de María Pastora, La Hembra,* and *Hierba de Maria.* Finally, the plant has been translated in English as “Leaves of the Shepherdess”, “Leaves of Mary Shepherdess”, “Sage of the Seers”, and “Diviners’ Sage”(Ott 1996). The origin of this plant is unknown. Though the Mazatec have been present in the Sierra Mazateca since pre-Hispanic times, it is unclear whether *S. divinorum* is a wild plant native to the Sierra Mazateca, a cultigen developed by the Mazatec, or a cultigen of another indigenous groups, perhaps of Mexica or Aztec origin, which was brought to the Sierra Mazateca and cultivated. In the present day, *S. divinorum* has not been discovered growing outside o the Sierra Mazateca, and indigenous groups of the Sierra Mazateca are the only people known to use *S. divinorum* in traditional ceremonies. However, Emboden suggested that the plant may be depicted in ancient Aztec murals (Ott 1996), and could in fact be the mysterious hallucinogen *pipiltzintzintli* known as “little prince” (Wasson 1963). Support for this hypothesis exists in the linguistic naming of *S. divinorum*; the plant lacks a true indigenous, Mazatecan name, but is included in a family of similarly pharmaco-religious hallucinogens; *Salvia divinorum* is known as *la hembra*, "the female" *el macho*, or "the male" is *Coleus pumila*, followed by *el nene*, "the child," and *el ahijado*, "the godson," which are both forms of *Coleus blumei* (Wasson 1962). Both *Coleus* species are Asiatic introductions, which Ott contends strengthens the argument that *S. divinorum* is nonnative (1996). Another hypothesis is that *ska Maria* is indeed native to the Sierra Mazateca region, but that the pastoral, shepherdess image of the Virgin depicted in many common names (such as *ska Pastora*) represents a remnant of the pre-Hispanic animal god worship, since Mary is not traditionally considered a shepherdess in Christianity (Wasson 1962). It is also unclear from many *S. divinorum* populations whether the plant is a cultigen or wild: while the plant is not grown in home gardens, *curanderos* seem to grow or encourage it in secret grottos or ravines near their villages, and “wild” populations found

by Reisfield could very possibly be vegetative clones surviving from a historic planting by indigenous peoples (1993).

The Mazatec are very protective of their knowledge regarding the use of ritual hallucinogens, and as a result, ethnobotanists have been challenged in their attempts to form a comprehensive understanding of pharmaco-religious hallucinogens in these traditional societies. Ethnobotanists have found in their research experience that it may be more fruitful to carefully develop a trusting relationship with one or two *curanderos* rather than interviewing many. While this method may skew the information somewhat, it has been most effective in producing the bulk of knowledge about Mazataec ethnobotany. The few willing informants, *curanderos* or *curanderas* who have risk committing sacrilege an desecration in allowing outsiders to witness the sacred ceremonies (Valdes 1983). Wasson described how the *curandera* María Sebastiana Carrera detailed the use of the leaves and repeated some of the ceremonial chants, but would not admit the researchers to an actual ceremony. After supplying them with this information, she broke out in tears, begging the heavens for forgiveness for revealing her knowledge (Wasson 1962). Hofmann further describes how the team members were taken in secret, at night, to the house of Consuela Garcia, where she performed a divination ceremony for them, concerned all the while that they would be discovered (Hofmann 1990). Ethnobotanists have learned much about the ethnobotanical uses of *S. divinorum* in curing, divination, and shamanic training among the Mazatec Indians, but mysteries still exist.

Many *Salvia* species are used throughout the world to cure; even the genus name comes from the Latin *salvare,* meaning “to save” (Valdes 1983). Though it may not be the primary role of *S. divinorum*, the plant is used medicinally by the Mazateca. Small dose infusions made from four to five pairs of leaves may be taken as a tonic or panacea (Valdes 1987). Taken in this form, *S. divinorum* is thought to regulate eliminatory functions, relieve headaches, and alleviate rheumatism. It may also given to the sick and dying to revive them. The Mazatec also believe that *ska Maria* will cure *panzon de barrego* (sic), or a swollen belly, which is supposedly caused by a curse from a *brujo*, or evil sorcerer. The victim's stomach swells up due to a "stone," but the *ska Maria* causes elimination of this "stone," curing the victim of the disease (Valdes 1983).

Maria Sabina, the *curandera* who informed Wasson on the uses of hallucinogenic mushrooms, mentions in her autobiography that crushed *hojas de la Pastora* could be used in place of mushrooms to cure a sick person, if mushrooms were not available (Valdes 1983). To use as a curative, *S. divinorum* is usually crushed by hand or in a *metate* and steeped in water, and depending on the illness, either the victim, the shaman, or both may take the infusion (Weitlaner 1959 in Valdes 2001a).

Healing and divination are closely linked in the Mazatec usage of *S. divinorum*. Shamanic hallucinogens of the Mazatec are almost always prescribed in pairs, which represents the human element of man and woman, symbolizing the dual principle of creation and procreation (Munn 1973). Weitlaner conducted interviews in which a native Mazatecan described healers using the leaves of the *Yerba de Maria* to divine illness when the cause was unknown. 50 leaves would be prescribed for a normal person, but 100 leaves would be prescribed for an alcoholic (reasons were not stated); the leaves would then be squeezed in water, and the sick person drank the potion in a dark, quiet place, such as a house. Then, after 15 minutes, the sick person would describe the illness from which he or she suffered. Then, at daybreak, the sick person would be bathed in the water they drank, thus releasing the person from the effects of the *Yerba de Maria* and curing them of the illness. Also, the native mentioned that robbery or loss could be divined by giving the person whose items were missing *Yerba de Maria*, and the *curandero* would then listen to them speak in trance to discover the cause (Weitlaner 1952, in Valdes 2001a). *S. divinorum* is also used by *curanderos* to foretell the future and answer questions about distant enemies, family or friends (Hofmann 1979, Valdes 1983). Hofmann describes a ceremony conducted by a *curandera* Consuela Garcia, attended by himself, Gordon Wasson, and Hofmann's wife Anita. While Hofmann did not participate due to illness, all other adults, including the *curandera*, took infusions of *S. divinorum* made by crushing 3 to 6 pairs of leaves in a *metate* (stone grinder) and squeezing them through a fine sieve. The infusions were incensed with copal "with great ceremony," before the *curandera* asked them if they believed in the holiness of Christ and the ceremony in which they would participate. Upon answering in the affirmative, the took they infusions. After chanting and singing, Consuela

Garcia asked them what information they wanted to know, and Wasson asked after the health of his daughter and new granddaughter in New York. The *curandera* responded that mother and child were well and healthy. Incidentally, the information was true, though neither Wasson or Hofmann suggest this as support for the *curandera's* divination (Hofmann 1979). Wasson witnessed a similar ceremony conducted by Augustina Borja in Ayuatla (Wasson 1962). In traditional ceremonies involving native Mazatecs, details in the ceremonies may differ. For instance, the Mazatecs are accustomed to chewing the leaves of *S. divinorum* directly, but researchers found this impossible due to the extremely bitter flavor of the leaves (Wasson 1962, Valdes 1983). Also, there is enormous variability in the number of leaves proposed for dosage in divination. Dosages could be as low as 6 leaves (Hofmann 1979) to as high as 120 pairs of leaves (Valdes et al 1983), though most reports seem to indicate that dosages are common in the 10-50 pair range (Wasson 1962, Valdes et al 1983).

Finally, *S. divinorum* is used in the training of new shamans among the Mazatec Indians. *Curanderos* and *curanderas* are made through informal apprenticeships, but believe that their true teaching comes through a series of visions showing or originating from heaven. Training can last 2 years, or longer, and involves the progressive use of psychotropic plants and mushrooms taken at intervals ranging from one week to one month; also, shamans may adopt a special diet before they take the hallucinogens. *S. divinorum* is the hallucinogen which begins a shaman's training, to "show him/her the way to heaven." Next, the student is exposed to morning glory seeds (*Rivea corymbosa (L.), Hallier, f.*) Hallucinogenic mushrooms containing the hallucinogen psilocybin are the final hallucinogen used to teach healers. This progression of psychotropic plants is based upon the strength of the effects: *S. divinorum* is the weakest of the three hallucinogens, generally producing a mild experience which can be terminated by noise or light. Morning glory seeds have more intense effects similar to LSD, though they also cause drowsiness and torpor. Finally, the pychotropic mushrooms employed by the Mazatec are used only as a final step, because the mushrooms have a "dark or sinister side;" apparently visions can be frightening, and the effects cannot be controlled or resisted. The Mazatecs contend that misuse of the mushrooms can lead to madness. The Mazatec believe that the visions they

experience from taking the sacred drugs allow them to contact Mary, The Trinity, and the Saints, who show them the different medicinal plants and teach them about their usage and treatments (Valdes et al 1983).

Biochemistry of Salvia divinorum

In the past two decades, most research concerning *S. divinorum* has focused on the psychoactive chemical compounds and its pharmacologic potential. *S. divinorum* has proven to be unique not only in its botanical significance and ethnopharmacological tradition, but also in its biochemical characteristics. Like many other members of the genus *Salvia*, *S. divinorum* contains unusual terpenoid compounds (Ott 2001).

In 1962, Gordon Wasson and Albert Hofmann collected *S. divinorum* juice from a leaves given to them in San Jose Tenango from the same flowering plants used as identification by Epling and Jativa.. The juice was preserved in alcohol to be studied, but chemical investigation at the time was unsuccessful. Hofmann concluded that the psychoactive principle must be unstable (Hofmann 1979). Diaz et al. also studied the mint, but were also had limited success in studying the chemical properties of the plant (Valdes 1994) .

S. divinorum's hallucinogenic components were isolated in 1982 by the research group of Alfredo Ortega, who had also isolated the new compounds salviarin and splendidin from *S. splendens* (Ott 2001). Ortega et al. isolated a novel trans-neoclerodane diterpene from *S. divinorum* and determined its structure using X-ray crystallography, but did not study biological activity, or extend the research to investigate the pharmacological applications of the compound. The new compound was named salvinorin (Ortega 1982).

L.J. Valdes has produced a large body of work regarding *S. divinorum*, both in ethnobotany and chemistry, and was also the first to test salvinorin as a psychoactive principle. In 1984, Valdes et al. isolated the same compound as Ortega et. al. Unaware that the compound had already been characterized and named, the group referred to active compound as divinorum A, and its inactive desacetyl derivative was called divinorum B. The two terms (salvinorin and divinorin) are now applied interchangeably, although salvinorin A and salvinorin B are,

officially, the correct names for these molecules (Valdes 1987, 1984). Valdes et. al. tested the biological effects of salvinorin A in mice, and noted that salvinorin had similar effects to mescaline, dramatically reducing animal activity in a manner similar to sedation, but without true sedation since the mice were able to move rapidly for short periods of time. The absolute stereochemistry of the salvinorins was also determined (Koreeda et al. 1990). While Valdes, Diaz, and Paul had personally tested *Salvia divinorum* leaves during Mazatec cermonies while conducting ethnobotanical research, Valdes et. al. did not conduct psychonautic human bioassays to determine whether salvinorin A was, in actuality, the visionary principle active in *S. divinorum* (Ott 1996).

"Basement shamans" (apparently Daniel Siebert and friends) in California were the next group to isolate and test salvinorin as a psychoactive principle (Ott 1996). Siebert soon tested various methods of leaf and salvinorin intake by volunteers to determine site of absorption, effects, and dosage. Siebert found that extended exposure (10 minutes) to the oral mucosa produced psychoactive effects in all volunteers, while quick swallowing and rinsing of leaves produced no effects at all, leading Siebert to conclude that the gastrointestinal system breaks down the psychoactive compound and that leaves must be chewed or held in the mouth to produce hallucinations. Siebert isolated salvinorin A by the same method used by Valdes et al. (1984). 20 volunteers were given capsules of salvinorin A, which produced no effects, reinforcing the hypothesis that salvinorin is inactivated by gastrointestinal absorption. Because salvinorin A is not water soluble, injection was not tested. Inhalation of the vaporized salvinorin A was tested, however, and proved to be the most efficient and dramatic method of salvinorin A intake to produce hallucinations. Threshold effects were usually noted at 200-500 Φg, and hallucinations occurred within 30 seconds, rather than the 10-15 minutes required by oral ingestion (Siebert 1994). With activity apparent at the 200 Φg level, salvinorin A is now the most potent entheogen known thus far, and one of the most potent natural compounds discovered (Valdes 2001, Ott 1996). Samples of salvinorin A were also submitted to Novascreen for receptor site screening, and was shown not to affect any brain receptor sites affected by most

other hallucinogens, suggesting that a unique pathway and receptor site may be present for salvinorin A (Siebert 1994). Salvinorin A is the first diterpene to be identified as a hallucinogen in humans (Valdes et al 2001). Siebert has also determined and compared levels of salvinorin A found in leaves from several plants grown throughout the United States and Mexico, concluding that leaves may contain a range of .89-3.70 mg/g salvinorin A in dry weight (Siebert 1999). Valdes et al. have further studied the bioactive compounds of *Salvia divinorum*, and discovered that a third compound exists, salvinorin C, which comprises only about 10% of the bioactive compounds in *S. divinorum*, but may be even more potent per unit of measure than salvinorin A (Valdes et al 2001).

Salvinorin A, salvinorin B, and salvinorin C bear close resemblance to other neoclerodane diterpenes from Latin American *Salvia* species, such as salviarin and splendidin. The salvinorins have been the only neoclerodane diterpenes tested for hallucinogenic properties thus far, however, and other similar *Salvia* compounds should be tested for pychotropic activities (Valdes et al 2001). Great research potential exists in the biochemistry and application of the salvinorin compounds, and related molecules, as hallucinogens, antibiotics, and to discover new neurological pathways and receptor sites.

The Future of *Salvia divinorum*

Since its introduction to the scientific community, *Salvia unknown* has proven to be a fascinating and enigmatic plant. While science has begun to unravel some of the many secrets surrounding *Salvia unknown*, both ethnobotanically and biochemically, *Salvia divinorum* is still very new to the general public. As a newcomer among western drugs, the role of *Salvia* as a recreational or pharmacological drug is, as of yet, undefined.

Until recently, *S. divinorum,* or “diviner’s sage” was not popular as a recreational drug in western society. *Salvia divinorum* has occasionally been used for some time outside of the Mazatec Indians as a recreational drug among Mexican teenagers, who purchase the dried leaves and smoke them as a replacement for marijuana (Valdes 1987). However, a number of factors discouraged the plant from greater popularity: a large number of fresh leaves are required to

obtain an intense experience (about 75-100 leaves). Once consumed, fresh *S. divinorum* leaves have an extremely bitter taste, which may induce vomiting. Even the effects of *S. divinorum* have been considered unpredictable or disappointing (Valdes 1994, Siebert 1994b).

Several developments have boosted the popularity of *Salvia divinorum* within the last decade. Young adults and adolescents have returned to entheogens as "natural highs," or to "expand consciousness." Moreover, the internet has made *S. divinorum* widely and rapidly available worldwide. Experimental drug users can now find an overwhelming amount of information and sales on webpages (Schabner 2002, pers. obsv.) *S. divinorum* is still completely legal everywhere in the world except Australia, where it was listed as illegal in November 2001 (Erowid 2002). While narcotics control groups in major nations worldwide are beginning to monitor *Salvia divinorum* closely, there are few movements to list the plant as illegal (Erowid 2002, Schabner 2002).

While the role of *Salvia divinorum* seems to have some parallels to other vision-inducing drugs such as LSD, it also has many differences which may prevent it from becoming a recreational drug similar to cocaine, marijuana, or LSD. Firstly, "diviner's mint" is not a social drug; it cannot be used to effect in distracting surroundings (Siebert 2002, Erowid 2002). Since it is not a party drug, *Salvia divinorum* is immediately more isolated to individual experimentalists. For *S. divinorum* to produce rewarding visions, users often find it best to smoke the dried leaves in a quiet, darkened room or to wear a blindfold, in order to experience the hallucinations at their fullest. Accounts of experiences from taking diviner's sage range from blissful to mystic to terrifying (Schabner 2002). Hallucinations can be interrupted by light, noise, and activity. Furthermore, there is no evidence that the drug is addictive, and the nature of the experience does not encourage users to repeat usage on a regular basis (Siebert 1994).

The effects of *Salvia divinorum* on the user vary greatly with setting and expectation. Valdes, Diaz, and others experienced *S. divinorum* usage in a religious, visionary ceremony with a Mazatec shaman, and thus had visions of the Virgin Mary and white-robed spirits (Valdes 1983). Milder trips often include visions of bright colors or changing shapes, while more intense experiences may produce hallucinations of being in another time and space, of flying or floating,

or speaking to strange beings (Siebert 2002, 1994). Drug users well accustomed to other hallucinogens often try the plant, appreciate the experience, but have no wish to repeat it . While the effects of *S. divinorum* seem to range from the mundane to the overpowering, no side effects or health problems have been known to occur from *Salvia divinorum* usage thus far (Schabner 2002, Siebert 1994). The danger in *Salvia* usage comes from the more intense experiences at higher dosages (500-1000 mcg salvinorin A), when the user may completely lose awareness and control over their body. People on *S. divinorum* may get up, lunge around the room, and attempt to walk through or over objects. It is necessary for *Salvia divinorum* users to have a sober and attentive sitter present to watch over their actions, particularly if the user intends to experience strong visions at high dosages (Siebert 1994, Campbell 1997, Valdes 1994).

S. divinorum appears to present few health risks either to individuals or the general public (Schabner 2002). The mechanism for psychoactivity with salvinorin A is completely unknown as of yet, since it binds to no known receptor sites (Siebert 2002). Therefore, the long-term health risks and effects of *Salvia divinorum* usage have not been studied. A group submitting a request to the DEA to maintain legal status of *Salvia divinorum* made a survey of emergency room records from across the country and found no records of treatment due to *Saliva divinorum* usage. No fatal overdoses are known to have occurred from *Salvia divinorum* or salvinorin A, although high doses may cause some unconsciousness and memory loss. If smoked (the most common method of consumption), experiences with *S. divinorum* are generally relatively short, lasting a few hours at most (Siebert 2002). However, doctors warn that users should be particularly careful not to mix salvinorin A with other drugs, and should not use *S. divinorum* if they, or their family, have a history of mental disorders, since hallucinations may trigger mental instability (Schabner 2002, Siebert 2002).

The pharmacological and research implications of *Salvia divinorum* are many and varied. Great potential exists for research with *Salvia divinorum.* Salvinorin A does not bind to any common receptor sites in the brain, and studies to discover the new pathway may further knowledge about consciousness and the human brain (Schabner 2002, Valdes 1994). *Salvia divinorum* may also produce studies in psychology. In one case report, a patient was found to

have found relief from symptoms of chronic depression after years of conventional treatment. The patient discovered *Salvia divinorum* independently (on the internet), but found that weekly, low dosage treatment helped her find a "psychospiritual" awakening which alleviated her depression (Hanes 2001). Rovinsky also found that acetone-soluble compounds from *Salvia divinorum* inhibited the growth of rod-shaped bacteria and decreased the duration of smooth muscle contraction in mice (1998). These findings suggest that *Salvia divinorum* may have several pharmacological applications that should be further researched.

In conclusion, the role of *Salvia divinorum* in the United States and other countries worldwide will depend greatly on societal perception of the plant as a drug in the near future. Already the media has begun to title *Salvia divinorum* as the "Hip New Drug," "The New LSD," which may sensationalize *Salvia divinorum* and increase the likelihood that it will be scheduled as an illegal narcotic. However, responsible use of *Salvia divinorum* may counter the media, particularly if the plant continues to affect only a small portion of experimental drug users. If so, *Salvia divinorum* may remain as a legal hallucinogen, set apart from other narcotics and pharmacological drugs by its ethnobotanical role, its unique attributes, and may someday provide new and beneficial uses in health and spiritual awareness.

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Divinatorins A−C, New Neoclerodane Diterpenoids from the Controlled Sage *Salvia divinorum*

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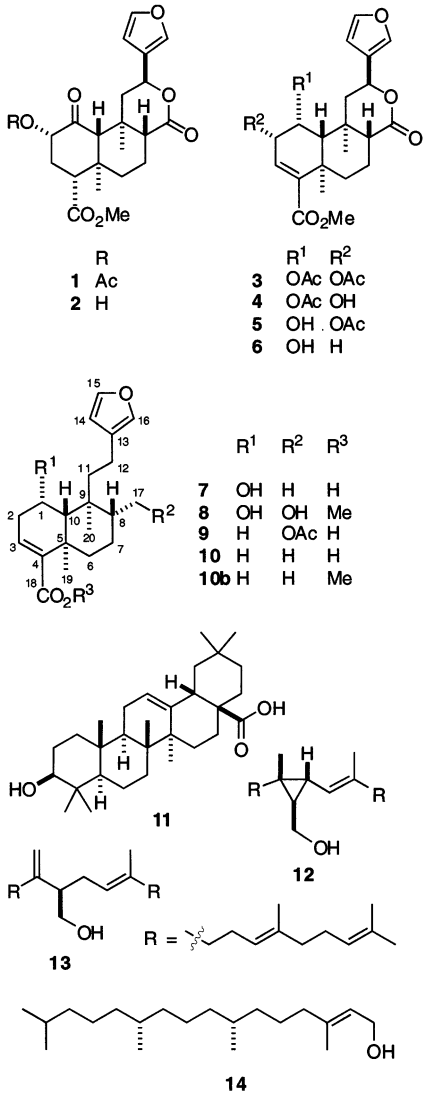
Three new neoclerodane diterpenoids, divinatorins A−C (**7**−**9**), have been isolated from the leaves of *Salvia divinorum*. The compounds were identified by spectroscopic methods as derivatives of the antibiotic (−)-hardwickiic acid (**10**), which was also isolated, along with four other known terpenoids. Neither the crude extract nor **7**−**9** displayed antimicrobial activity.

Salvia divinorum Epling & Játiva (Lamiaceae) is a sage used medicinally by the Mazatec Indians of Oaxaca, Mexico. The leaves contain salvinorin A (**1**),[1] a potent hallucinogen acting at the kappa opioid receptor.[2] Recently, the plant has gained notoriety as a legal hallucinogen, sold openly on the Internet. As a result, the plant and compound **1** have been prohibited in Australia[3] (a recent bill to prohibit the plant in the United States was not enacted).[4] The enforcement of such controls is likely to be hampered by the plant's nondescript appearance and the very limited chemical data available.[5] Until recently, only four compounds had been isolated from this species: salvinorins A (**1**), B (**2**),[6] and C (**3**)[7] and loliolide.[8] GC/MS analysis also indicated the possible presence of neophytadiene and stigmasterol.[5]

Recently we reported the isolation of salvinorins D−F (**4**−**6**) from the acetone extract of commercial *S. divinorum*,[9] employing chromatography on activated carbon to separate the terpenoids from complicating pigments. This work generated several mixed fractions, which appeared initially to be inseparable. Exhaustive chromatography on silica gel, employing high silica ratios and diverse solvent systems, has now yielded divinatorins A−C (**7**−**9**), along with the known terpenoids (−)-hardwickiic acid (**10**)[10] and oleanolic acid (**11**).[11]

In addition, an extraction of locally grown leaves was undertaken. The acetone extract was again chromatographed on activated carbon; elution with EtOAc/petrol gave partial separation of two terpenoid fractions. The first, after chromatography on silica gel, yielded the known terpenoids presqualene alcohol (**12**),[12] peplusol (**13**),[13] and (*E*)-phytol (**14**).[14] The second mixture was recrystallized from MeOH to give **1**, albeit in much lower yield than from the commercial material (0.56 g/kg).

The structures of **7**−**9** were elucidated chiefly by NMR (^{1}H,^{13}C, DEPT, HMQC, HMBC, COSY, and NOESY in each case). The ^{1}H NMR spectra suggested that they were derivatives of **10**. The molecular formula of **7**, $C_{20}H_{28}O_4$ (HRESIMS), implied the presence of a single hydroxyl substituent, which was confirmed by an IR absorption at 3392 cm^{-1}. This was located at C-1 on the basis of 2D NMR: the oxymethine at δ 4.49 showed couplings to H-2 and -10 (COSY) and C-3, -9, and -10 (HMBC). The configuration was confirmed by an H-1 to H-11 cross-peak in the NOESY spectrum.



The ^{1}H NMR spectrum of **8** suggested a methyl ester (δ 3.71) with two hydroxyl groups [δ 1.49 (2H, D_2O-exchangeable)]. A strong IR absorption band occurred at 3434 cm^{-1}. The molecular formula, $C_{21}H_{30}O_5$ (HRESIMS), was consistent with this proposal. One of the hydroxyl groups was again located at C-1, showing the same couplings as in **7**. The second was located at C-17, on the basis of the couplings of the oxymethylene signals (δ 3.38 and 3.84) to H-8 (COSY) and to C-7, -8, and -9 (HMBC). The NOESY

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Table 1. ^{1}H NMR Data (400 MHz) for Compounds **7**−**9**[a]

	δ, m, J (Hz)		
H	**7**	**8**	**9**
1	4.49 br d (4.8)	4.46 br d (4.9)	1.70 m
			1.46 m
2	2.56 ddd (20.1, 5.1, 2.8)	2.53 ddd (19.9, 5.1, 2.8)	2.35 dt (20.5, 5.1)
	2.40 m	2.34 m	2.19 m
3	6.90 dd (4.8, 2.7)	6.65 dd (4.8, 2.7)	6.89 dd (4.4, 2.9)
6	2.40 m	2.36 m	2.53 dt (13.2, 3.2)
	1.20 m	1.16 m	1.15 td (13.2, 3.6)
7	1.57 m	1.87 m	1.74 m
	1.43 m	1.56 m	1.48 m
8	1.55 m	1.58 m	1.79 m
10	1.45 br s	1.45 br s	1.42 br d (12.1)
11	1.85 ddd (14.7, 12.8, 4.7)	1.91 m	1.75 m
	1.68 m	1.77 m	1.63 m
12	2.33 m	2.42 td (13.6, 4.6)	2.40 td (13.8, 4.2)
	2.05 ddd (14.3, 12.9, 4.7)	2.08 ddd (14.1, 12.8, 4.7)	2.20 m
14	6.25 br s	6.25 br s	6.28 br s
15	7.36 t (1.6)	7.35 t (1.7)	7.35 t (1.6)
16	7.20 br s	7.20 br s	7.22 br s
17	0.84 d (6.0)	3.84 dd (10.5, 3.7)	4.26 dd (11.0, 4.1)
		3.38 dd (10.5, 8.0)	3.79 dd (11.0, 8.4)
19	1.64 s	1.66 s	1.27 s
20	1.15 s	1.18 s	0.83 s
CO_2CH_3		3.71 s	
$OCOCH_3$			2.03 s
OH		1.49 br s	

[a] In $CDCl_3$ as solvent and internal standard (7.26 ppm).

spectrum showed H-1 to H-11 and H-17 to H-20 cross-peaks, confirming the configuration at these centers.

The ^{1}H NMR spectrum of **9** showed an acetyl methyl signal (δ 2.03). The oxymethylene signals, shifted downfield to δ 3.79 and 4.26, showed the same couplings as in **8**, establishing the 17-acetoxy structure shown. This was consistent with the molecular formula, $C_{22}H_{30}O_5$ (HRESIMS). The expected relative stereochemistry of compounds **7**−**9** was confirmed in each case by NOESY cross-peaks from H-20 to H-17 and -19 (setting C-5, -8, and -9), and H-10 to H-12 (setting C-10). The absolute stereochemistry shown is common to all clerodanes isolated from the Lamiaceae,[15] including **1**[16] and **10**.[17]

Since compound **10** has previously been shown to display potent, broad-spectrum antimicrobial activity,[10] **7**−**9** were screened against standard antibiotic-susceptible strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*, using standard microdilution[18,19] and disk diffusion[20] assays. Compounds **7**−**9** showed no activity against any of the test organisms at 100 μg/mL or 100 μg/disk. These data extend the remarkably stringent structure−activity requirements of **10**.[10] To probe this further, we decided to screen (+)-hardwickiic acid (*ent*-**10**). This was isolated from copaiba balsam as the methyl ester.[21] Hydrolysis proved challenging: reflux in KOH/MeOH gave only slow decomposition, but microwave irradiation on KF/Al_2O_3[22] provided *ent*-**10** in low yield. *ent*-**10** proved active against *S. aureus* (MIC 25 μg/mL) and *B. subtilis* (MIC 12.5 μg/mL, 10 mm zone of inhibition), but much less potent than its enantiomer (MIC 0.78 μg/mL against *B. subtilis*).[10]

A previous non-peer-reviewed investigation[23] reported the acetone extract of *S. divinorum* to be active against a wide range of bacteria. We were unable to confirm these results. The acetone extract of the commercial material, as well as **1**, showed no activity at 100 μg/mL or 100 μg/disk. Probably insufficient **10** and **11** were present to elicit an effect (**11**, like **10**, is active against *B. subtilis* and *S. aureus*).[24]

Experimental Section

General Experimental Procedures. Instruments and materials were as described previously.[9] Flash column chromatography was performed on Merck silica gel 60. Silica:solute mass ratios up to 400:1 were used for difficult separations (ΔR_f < 0.05). Vacuum chromatography was performed on Merck activated carbon 2183. 'Petrol' refers to the petroleum ether fraction boiling at 40−60 °C.

Plant Materials. *S. divinorum* plants, cultivated in Melbourne, were harvested in February 2003. A voucher specimen was deposited at the National Herbarium of Victoria (accession number MEL 2145478). Copaiba balsam was donated by Australian Botanical Products (Hallam, Victoria).

Antimicrobial Tests. The crude extract and pure compounds were tested against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6633), and *Candida albicans* (ATCC 90028) using standard broth microdilution[18,19] (100−0.19 μg/mL using 2-fold serial dilutions) and disc-diffusion[20] assays (100 μg/disk). All measurements were performed in duplicate. Streptomycin sulfate and amphotericin B were used as positive controls.

Extraction and Isolation. Dried commercial *S. divinorum* leaves (860 g) were extracted as described previously.[9] The mother liquor from recrystallization of **1** was subjected to flash column chromatography (FCC) on silica gel in a 5−50% acetone/CH_2Cl_2 gradient. This was divided based on TLC (10% acetone/CH_2Cl_2) into four series: A (656 mg), B (150 mg), C (359 mg), and D (77 mg).

Series A: 90 mg was subjected to FCC, eluting with a gradient from 50 to 80% Et_2O/petrol, to give **10** (6 mg).

Series C: trituration in Et_2O gave **4** (75 mg). FCC of the mother liquor (60−100% Et_2O/petrol) gave four fractions based on TLC (70% Et_2O/petrol): C1 (55 mg), C2 (119 mg), C3 (57 mg), and C4 (26 mg)

Fraction C1: repeated FCC (20% acetone/petrol and 40−60% Et_2O/petrol) gave **9** (23 mg) and **11** (3 mg).

Fraction C2: repeated FCC (25% acetone/petrol and 60−100% Et_2O/petrol) gave **8** (32 mg).

Fraction C3: extensive FCC (Et_2O/petrol, acetone/petrol, and EtOAc/petrol) gave additional **8** (total yield 41 mg) and a mixture of **5** and **6**, which were separated as described previously.[9]

Fraction C4 gave pure **4** (total yield 114 mg).

Table 2. ^{13}C NMR Data (100 MHz) for Compounds **7**−**9**[a]

	δ		
C	**7**	**8**	**9**
1	64.7	64.3	17.0
2	38.1	38.0	27.4
3	136.2	133.2	140.3
4	140.8	141.4	141.2
5	37.4	37.1	37.4
6	38.6	38.0	35.2
7	27.4	21.9	22.3
8	37.1	44.8	40.9
9	39.7	39.1	38.4
10	49.0	48.7	46.8
11	39.1	38.8	38.9
12	18.2	18.2	18.3
13	125.2	124.9	125.2
14	110.9	110.8	110.9
15	142.8	142.8	142.8
16	138.4	138.4	138.5
17	15.7	63.9	66.1
18	171.8	167.3	171.9
19	21.4	21.4	20.5
20	19.8	20.9	19.0
CO_2CH_3		51.3	
$OCOCH_3$			21.0
$OCOCH_3$			171.2

[a] In $CDCl_3$ as solvent and internal standard (77 ppm).

Series D: repeated FCC (60% Et_2O/petrol and 4% MeOH/CH_2Cl_2) gave **7** (36 mg).

Extraction of Australian Material. Dried, powdered Australian-grown *S. divinorum* leaves (224 g) were steeped in acetone for 30 min (3 × 250 mL). Filtration and evaporation under reduced pressure gave a dark green tar (7 g). This was purified by vacuum column chromatography on a mixture of activated carbon (75 g) and diatomite filter aid (~1:1), eluting with a gradient from 50 to 20% EtOAc/petrol, to give series E (97 mg) and F (279 mg) based on TLC (70% Et_2O/petrol).

Series E: repeated FCC (1% acetone/CH_2Cl_2 and 20% Et_2O/petrol) gave **13** (6 mg). Further FCC (0.75% MeOH/CH_2Cl_2 and 1% EtOH/$CHCl_3$) gave **12** (23 mg) and **14** (12 mg).

Series F: two recrystallizations from MeOH gave **1** (126 mg).

Divinatorin A (7): amber resin; $[\alpha]^{19}_D$ −53° (*c* 1.8, CH_2Cl_2); FTIR (film) ν_{max} 3392, 2927, 2874, 2648, 1684, 1456, 1411, 1386, 1245 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS [M + Na^+] *m*/*z* 355.1864 (calcd for $C_{20}H_{28}O_4Na^+$, 355.1880); TLC, see Table S1.

Divinatorin B (8): amber resin; $[\alpha]^{20}_D$ −54° (*c* 2.1, $CHCl_3$); FTIR (film) ν_{max} 3434, 2930, 2881, 1714, 1437, 1236, 1067 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS [M + Na^+] *m*/*z* 385.1988 (calcd for $C_{21}H_{30}O_5Na^+$, 385.1985); TLC, see Table S1.

Divinatorin C (9): amber resin; $[\alpha]^{25}_D$ −110° (*c* 1.1, $CHCl_3$); FTIR (film) ν_{max} 2960, 2873, 1738, 1681, 1236, 1025 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS [M + Na^+] *m*/*z* 397.1989 (calcd for $C_{22}H_{30}O_5Na^+$, 397.1985); TLC, see Table S1.

Methyl (−)-hardwickiate (10b): amber syrup; $[\alpha]^{25}_D$ −115° (*c* 0.03, $CHCl_3$) [lit.[10] −104°]; ^1H and ^{13}C NMR, FTIR, and EIMS (70 eV) matched literature values.[10]

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Supporting Information Available: ^1H and ^{13}C NMR spectra for **7**−**9**; TLC data for **1**−**14**; isolation procedure for *ent*-**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Salvinorin A, an Active Component of the Hallucinogenic Sage *Salvia divinorum* Is a Highly Efficacious κ-Opioid Receptor Agonist: Structural and Functional Considerations

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ABSTRACT

The diterpene salvinorin A from *Salvia divinorum* has recently been reported to be a high-affinity and selective κ-opioid receptor agonist (Roth et al., 2002). Salvinorin A and selected derivatives were found to be potent and efficacious agonists in several measures of agonist activity using cloned human κ-opioid receptors expressed in human embryonic kidney-293 cells. Thus, salvinorin A, salvinorinyl-2-propionate, and salvinorinyl-2-heptanoate were found to be either full (salvinorin A) or partial (2-propionate, 2-heptanoate) agonists for inhibition of forskolin-stimulated cAMP production. Additional studies of agonist potency and efficacy of salvinorin A, performed by cotransfecting either the chimeric G proteins Gaq-i5 or the universal G protein Ga16 and quantification of agonist-evoked intracellular calcium mobilization, affirmed that salvinorin A was a potent and effective κ-opioid agonist. Results from structure-function studies suggested that the nature of the substituent at the 2-position of salvinorin A was critical for κ-opioid receptor binding and activation. Because issues of receptor reserve complicate estimates of agonist efficacy and potency, we also examined the agonist actions of salvinorin A by measuring potassium conductance through G protein-gated K^+ channels coexpressed in *Xenopus* oocytes, a system in which receptor reserve is minimal. Salvinorin A was found to be a full agonist, being significantly more efficacious than (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methanesulfonate hydrate (U50488) or (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methanesulfonate hydrate (U69593) (two standard κ-opioid agonists) and similar in efficacy to dynorphin A (the naturally occurring peptide ligand for κ-opioid receptors). Salvinorin A thus represents the first known naturally occurring non-nitrogenous full agonist at κ-opioid receptors.

Salvia divinorum, a member of the Lamiaceae family, has been used by the Mazatec Indians of northeastern Oaxaca, Mexico, primarily for its psychoactive effects (Wasson, 1962, 1963) for many hundreds of years (for reviews, see Valdes et al., 1983; Sheffler and Roth, 2003). The active ingredient of *S. divinorum* is salvinorin A, a non-nitrogenous neoclerodane diterpene that represents the most potent naturally occurring hallucinogen known (Valdes et al., 1984; Siebert, 1994). Salvinorin A induces an intense, short-lived hallucinogenic experience qualitatively distinct from that induced by the classical hallucinogens lysergic acid diethylamide, psilocybin, and mescaline (Siebert, 1994). Both *S. divinorum* and salvinorin A have been used recreationally for their hallucinogenic properties (Giroud et al., 2000). Intriguingly, an anecdotal case report has suggested that *S. divinorum* may have antidepressant properties as well (Hanes, 2001).

Quite recently, we discovered that salvinorin A has high affinity and selectivity for the cloned κ-opioid receptor (KOR) and suggested, based on limited functional studies, that salvinorin A was a KOR agonist (Roth et al., 2002). We now present a detailed report on the agonist properties of salvinorin A and selected derivatives. We discovered that salvinorin A is an extraordinarily efficacious and potent κ-opioid

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ABBREVIATIONS: KOR, κ-opioid receptor; hKOR, human κ-opioid receptor; nor-BNI, nor-binaltorphimine; U50488, (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methane-sulfonate hydrate; U69593, (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]*dec*-8-yl]-benzeneacetamide.

agonist. We also found, based on structure-function studies, that the nature of the substituent on the 2-position of salvinorin profoundly affects functional activity. Together, these results support the hypothesis that the unique effects of salvinorin A on human perception are due to selective activation of KOR.

Materials and Methods

Materials. U50488, U69593, dynorphin A, norbinaltorphimine (nor-BNI) were obtained from Sigma-Aldrich (St. Louis, MO). [^{3}H]Bremazocine was from PerkinElmer Life Sciences (Boston, MA).

Complementary DNA Clones and cRNA Synthesis for Oocyte Studies. The rat KOR was obtained from Dr. David Grandy (GenBank accession no. D16829). The human KOR cDNA was obtained from the Guthrie Research Foundation (GenBank accession no. NM000912) and subcloned into the eukaryotic expression vector pIRESNEO (Invitrogen, Carlsbad, CA); cDNAs for $K_{IR}3.1$ (accession no. U01071) and $K_{IR}3.2$ (accession no. U11859) were obtained from Drs. Cesar Lebarca and Henry Lester, respectively. The chimeric G protein Gq-i5 was obtained from Bruce Conklin (University of California, San Francisco), whereas Gα16 was obtained from the Guthrie Research Foundation; both constructs were verified by automated dsDNA sequencing (Cleveland Genomics, Inc., Cleveland, OH) before use. Plasmid templates for all constructs were linearized before cRNA synthesis, and the mMESSAGE MACHINE kit (Ambion, Austin, TX) was used to generate capped cRNA.

Cell Lines and Maintenance. A stable line expressing the human KOR (hKOR-293) was obtained by transfecting an hKOR expression vector (hKOR-pIRESNEO) into human embryonic kidney-293 cells (maintained and transfected as previously detailed; Roth et al., 2002) and selecting in 600 μg/ml G418. Surviving clones were expanded and characterized with one (hKOR-293) that expressed high levels of hKOR (ca. 1 pmol/mg) used for further studies.

Oocyte Maintenance and Injection. Healthy stage V and VI oocytes were harvested from mature anesthetized *Xenopus laevis* (Nasco, Ft. Atkinson, WI) and defolliculated enzymatically as described previously (Snutch, 1988). The oocytes were maintained at 18°C in standard oocyte buffer, ND96 (96 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5), supplemented with 2.5 mM sodium pyruvate and 50 μg/ml gentamicin (Sigma-Aldrich). One day after harvest, cRNAs were injected (50 nl/oocyte) with a Drummond microinjector. Each oocyte was injected with 0.5 ng of KOR cRNA and 0.1 ng of $K_{IR}3.1$ and $K_{IR}3.2$ cRNA. Recordings were made at least 48 h after injection.

Electrophysiological Studies. An Axon Geneclamp 500 amplifier was used for standard two-electrode voltage-clamp experiments. The FETCHEX program (Axon Instruments, Foster City, CA) and recorded data traces were used for data acquisition and analysis. Oocytes were then removed from incubation medium, placed in the recording chamber containing ND96 medium, and clamped at −80 mV. Recordings were made in hK buffer (72.5 mM NaCl, 24 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5). To facilitate the recording of inward K^+ currents through the $K_{IR}3$ channels, the normal oocyte saline buffer was modified to increase the KCl concentration to 24 mM K^+. Microelectrodes were filled with 3 M KCl and had resistances of 0.4 to 2.0 MΩ.

Radioligand Binding and Functional Studies. Radioligand binding studies were performed as described previously (Roth et al., 2002) with the exception that 150 mM NaCl was added to the standard binding buffer to mimic physiological sodium concentrations. In brief, membranes (10–50 μg) were incubated together with [^{3}H]bremazocine in a final volume of 0.5 ml with a buffer of the following composition: 50 mM Tris-HCl, 150 mM NaCl, pH 7.40 along with test agents for 90 min at room temperature. Incubations were terminated by rapid filtration and collection on GF/C glass fiber filters and washing with ice-cold binding buffer. Dried filters were put into sample vials, scintillation fluid was added, and dpm were measured by liquid scintillation spectroscopy. Measurements of the ability of KOR agonists to inhibit forksolin-stimulated adenylate cyclase activity were performed as detailed previously (Roth et al., 2002). For studies involving measurements of intracellular calcium mobilization, a Molecular Devices FLEXSTATION was used as recently detailed (Rothman et al., 2003). For these studies, hKOR were cotransfected with the chimeric G protein Gaq-i5 (Conklin et al., 1993) or the "universal" G protein Ga16 (Offermanns and Simon, 1995). Measurements of intracellular calcium mobilization and quantification of agonist efficacy and potency were performed as described in Rothman et al. (2003).

Data Analysis. EC_{50} values and curve fitting were determined using Nfit software (Island Products, Galveston, TX) or GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Student's *t* test was used for comparison of independent means, with values reported as two-tailed *p* values.

Chemistry. Salvinorin A was isolated from dried leaves of *S. divinorum* by the method reported previously (Valdes et al., 1984). Salvinorin A was hydrolyzed using potassium carbonate in methanol to yield salvinorin B. The reported esters were formed using salvinorin B, dimethylaminopyridine, and the corresponding acid chloride in methylene chloride.

Salvinorin B was characterized by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry (HRMS) and found to be authentic by comparison with literature values (Valdes et al., 1984). The reported esters were purified by high-performance liquid chromatography and characterized by HRMS. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 500 MHz NMR spectrometer in $CDCl_3$. The HRMS were measured using a Bioapex FT mass spectrometer with electrospray ionization. High-performance liquid chromatography was conducted on a Waters Deltaprep 4000 system using a Waters Xterra RP_{18}, 5 μm, 4.6 × 150-mm column, with mobile phase H_2O/acetonitrile (1:1). Thin layer chromatography analyses were carried out on precoated Si gel G_{254}, 250-μm plates, with the developing system hexane/ethyl acetate (2:1) and visualized with vanillin/H_2SO_4 in ethanol.

Preparation of Esters. Salvinorin B (10 mg, 26 nmol) and 4-dimethylaminopyridine (catalytic amount) were dissolved in methylene chloride (3 ml). The corresponding acid chloride (130 nmol) was added, and the reaction stirred at room temperature overnight. The

TABLE 1
Calculated molecular weights were obtained using ChemDraw software

Yields and Masses of Salvinorinyl Esters		Calculated	Found$(M + 23)_{for\ sodium}$
1) Propionate	9.0 mg, 78.5%	446.1941	469.1917
2) Heptanoate	10.5 mg, 81.6%	502.2567	525.2566
3) Pivalate	11.1 mg, 91.4%	474.2254	497.2215
4) *p*-Bromobenzoate	12.4 mg, 84.4%	572.1046	595.1009
5) 2,2,2-Trichloroethylcarbonate	11.5 mg, 79.4%	564.0721	587.0689
6) Ethylcarbonate	9.8 mg, 82.7%	462.1890	485.1833
7) Piperonylate	1.6 mg, 11.6%	538.1839	561.1834
8) 1-Naphthoate	2.1 mg, 15.1%	544.2097	567.2087
9) Cyclopropanecarboxylate	10.5 mg, 89.4%	458.1941	481.1952

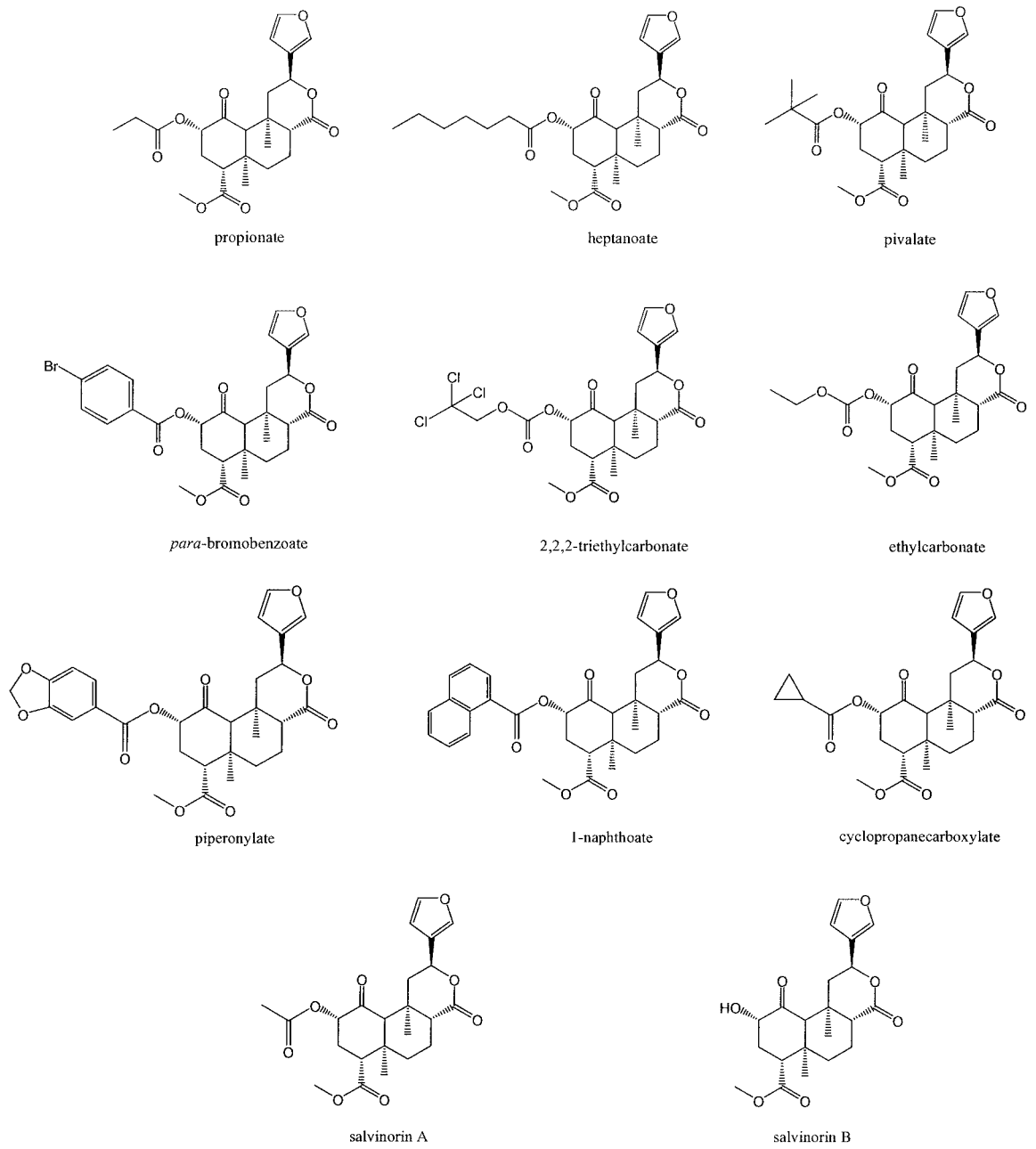

Fig. 1. Structures of salvinorin A, B, and 2-salvinorinyl esters. Shown are the structures of the compounds used in this study.

mixture was quenched with methanol, loaded onto silica, and purified by vacuum liquid chromatography using Si gel (230–400-mesh) with hexane/ethyl acetate (3:1) solvent system. Calculated molecular weights were obtained using ChemDraw software (Table 1).

Results

In initial studies, we examined the abilities of salvinorin A and selected derivatives (see Fig. 1 for structures) for their ability to bind to hKORs. As can be seen, the synthetic derivatives of salvinorin A differ solely in the nature of the substituent in the 2-position. As is shown in Table 2, salvinorinyl-2-propionate was the only derivative with submicromolar affinity for hKORs; also of note is that salvinorin B was inactive at hKORs. A screen of a number of other receptor subtypes showed that the salvinorin A derivatives tested had no significant activity at other receptors, including various serotonergic, dopaminergic, muscarinic, adrenergic, cannabinoid, and σ receptors (see Table 2 for details)

We next evaluated the ability of salvinorin A and the propionate and heptanoate derivatives to activate hKORs by measuring the ability to inhibit forskolin-stimulated cAMP production using U69593 as the comparator. As shown in Table 2, salvinorin A and salvinorinyl-2-propionate were potent and full agonists compared with U69593, whereas salvinorinyl-2-heptanoate was a partial agonist.

We also evaluated the ability of U69593, dynorphin A, salvinorin A, and the propionate derivative of salvinorin A to activate hKORs using a fluorescent-microplate-reader (FLEXSTATION) wherein hKORs were cotransfected with either the chimeric G protein Gqi5 or the universal G protein

TABLE 2

Effect of salvinorin A derivatives on KOR binding and inhibition of forskolin-stimulated adenylate cyclase in KOR-293 cells

Shown are the mean values ± S.D. from n = 2–4 separate experiments in which K_i values for inhibition of [^{3}H]bremazocine binding and EC_{50} and E_{max} values for inhibition of adenylate cyclase in KOR-293 cells were performed as detailed under Materials and Methods with the response induced by U69593 defined as 100%. The salvinorin A derivatives listed above were also screened at a large number of cloned receptors and found to have no significant activity, when tested at 10 μM at the following receptors: serotonin (5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3, 5-HT5a, 5-HT6, 5-HT7), dopamine (D1, D2, D3, D4, D5), muscarinic (m1, m2, m3, m4, m5), μ, δ, and ORL-1 opioid receptors, σ1, σ2, α1-adrenergic (1a, 1b, 1d), α2-adrenergic (2A, 2B, 2C) β2-adrenergic, and CB-1 cannabinoid receptors [assayed as previously detailed (Shi et al., 2003)].

	K_i ± S.E.M. (nM)	EC_{50} in nM (pEC_{50} ± S.E.M.)	E_{max}
Salvinorin A	18.74 ± 3.38	0.63 (−0.2 ± 0.07)	100
Propionate	32.63 ± 15.7	4.7 (0.7 ± 0.3)	100
Heptanoate	3199 ± 961.2	40 (1.6 ± 0.4)	34 ± 11
Privalate	>10,000	NA	NA
p-Bromobenzoate	>10,000	NA	NA
2,2,2-Triethylcarbonate	>10,000	NA	NA
Ethylcarbonate	>10,000	NA	NA
Piperonylate	>10,000	NA	NA
1-Napthoate	>10,000	NA	NA
Cyclopropanecarboxylate	>10,000	NA	NA
Salvinorin B	>10,000	NA	NA

NA, not active at 10,000 nM.

Ga16 as detailed previously (Rothman et al., 2003). Figure 2 shows representative results for U69593 and salvinorin A using either Gα16 (A and B) or Gq-i5 (C and D). No responses were seen in untransfected cells or in cells transfected with hKOR alone (data not shown). Figure 2 also shows a representative dose-response study using Gq-i5 as the chimeric G protein. Because both methods seemed to yield equivalent results, further studies were performed with Gq-i5. Table 3 shows representative EC_{50} and E_{max} values for a variety of KOR agonists using Gq-i5. In these studies, salvinorin A was more potent than any other of the tested KOR agonists (Table 3). In terms of maximal response, all of the active compounds gave similar responses.

It is well known that overexpression systems tend to provide inaccurate estimates of agonist potencies and efficacies because of issues of receptor reserve (Kenakin, 2002). As well, it has been well described that unnatural expression systems wherein chimeric or "universal" G proteins are used also lead to misleading estimates of agonist potencies and maximal responses (Woolf et al., 2001; Kenakin, 2002). Accordingly, we next determined the maximal agonist responses (E_{max}) and potencies (EC_{50} values) of selected compounds using a system without receptor reserve.

Salvinorin A Is a Full agonist. For these studies, *Xenopus* oocytes were coinjected with inwardly rectifying K^+ channels and KORs. In the experiment shown, a representative oocyte voltage clamped at −80 mV was first perfused with hK buffer (containing 24 mM KCl) to shift the reversal potential of potassium and facilitate K^+ current through Kir3 (Fig. 3). Perfusion with 1 μM salvinorin A significantly increased the inward current, and the activation was reversed by 100 nM nor-BNI. Similarly, 1 μM U69593 increased the inward current in a different oocyte, and the effect was also blocked by 100 nM nor-BNI (Fig. 1B). Neither 10 μM salvinorin A nor U69593 increased the membrane conductance of oocytes expressing Kir3 without KOR (data not shown).

Concentration-response curves of salvinorin-A and κ-agonists U69593 and U50488 were compared (Fig. 4). Each point represents the mean response measured in four to seven different oocytes. Data were collected from multiple batches of oocytes and merged by normalizing the responses to the average maximal response produced by salvinorin A on that recording day. Based on these results, salvinorin A was not significantly more potent (EC_{50} = 69 nM; confidence intervals 50–94 nM) than U69593 (EC_{50} = 224 nM; confidence intervals 51–157 nM) or U50488 (EC_{50} 150 nM; confidence intervals 50–194 nM).

Under these expression conditions, there was an apparent lack of spare κ-receptors. Increasing the κ-receptor cRNA from 0.5 ng/oocte to 1.0 ng increased the average U69593 response from 1.63 ± 0.57 to 2.76 ± 1.04 μA (n = 7 or 8). Based on the lack of spare receptors, we directly compared the maximal responses evoked by 10 μM each of the κ-agonists (Fig. 5) with that of dynorphin A. In this assay, propionyl-salvinorin also acted as a partial agonist whose maximal activity was less than salvinorin A. The response to salvinorin A was significantly greater than that to U69593 and U50488 ($p < 0.05$), but not significantly greater to that of dynorphin A.

Discussion

The principal finding of this study is that salvinorin A is an extraordinarily potent full agonist at hKORs. Additionally, we report that salvinorinyl-2-propionate is a potent partial agonist at KORs and also demonstrate that the nature of the 2-substituent of the salvinorin scaffold is critically important for agonist efficacy and potency. We also have obtained data with KOR-knockout and wild-type mice that the actions of salvinorin A are mediated by KOR in vivo (J. Pintar, personal communication). Together, these results imply that the profound effects of salvinorin A on human consciousness are mediated by potent and highly efficacious activation of KORs.

In prior reports, we have suggested that because salvinorin A is a potent hallucinogen that is apparently selective for KORs, and that targeting KORs might lead to novel medications for the treatment of diseases manifested by hallucinatory experiences (e.g., schizophrenia, affective disorders, and dementia) (Roth et al., 2002; Sheffler and Roth, 2003). In this regard, studies with nonselective opioid antagonists that possess KOR actions in schizophrenia have been mixed (Rapaport et al., 1993; Sernyak et al., 1998), although there are no studies in which selective KOR antagonists have been tested. Because of anecdotal reports that extracts of *S. divinorum* may possess antidepressant actions (Hanes, 2001), and published studies in rodents that KOR antagonists block stress-induced responses (McLaughlin et al., 2003), KOR antagonists could possess antianxiety/antidepressant actions as well. Indeed, a recent study (Mague et al., 2003) suggested that κ-selective antagonists might have intrinsic antidepressant actions. Our current studies suggest that novel KOR-selective agents might be obtained by selective modification of the salvinorin scaffold. Whether such agents might possess antidepressant or antipsychotic activity is unknown.

As shown in these studies, salvinorin A and salvinorinyl-2-propionate are potent agonists at KORs with salvinorin A being a full agonist in most assay systems, whereas salvinorinyl-2-propionate is likely a partial agonist. Salvinorin B and all other tested salvinorin derivatives were devoid of

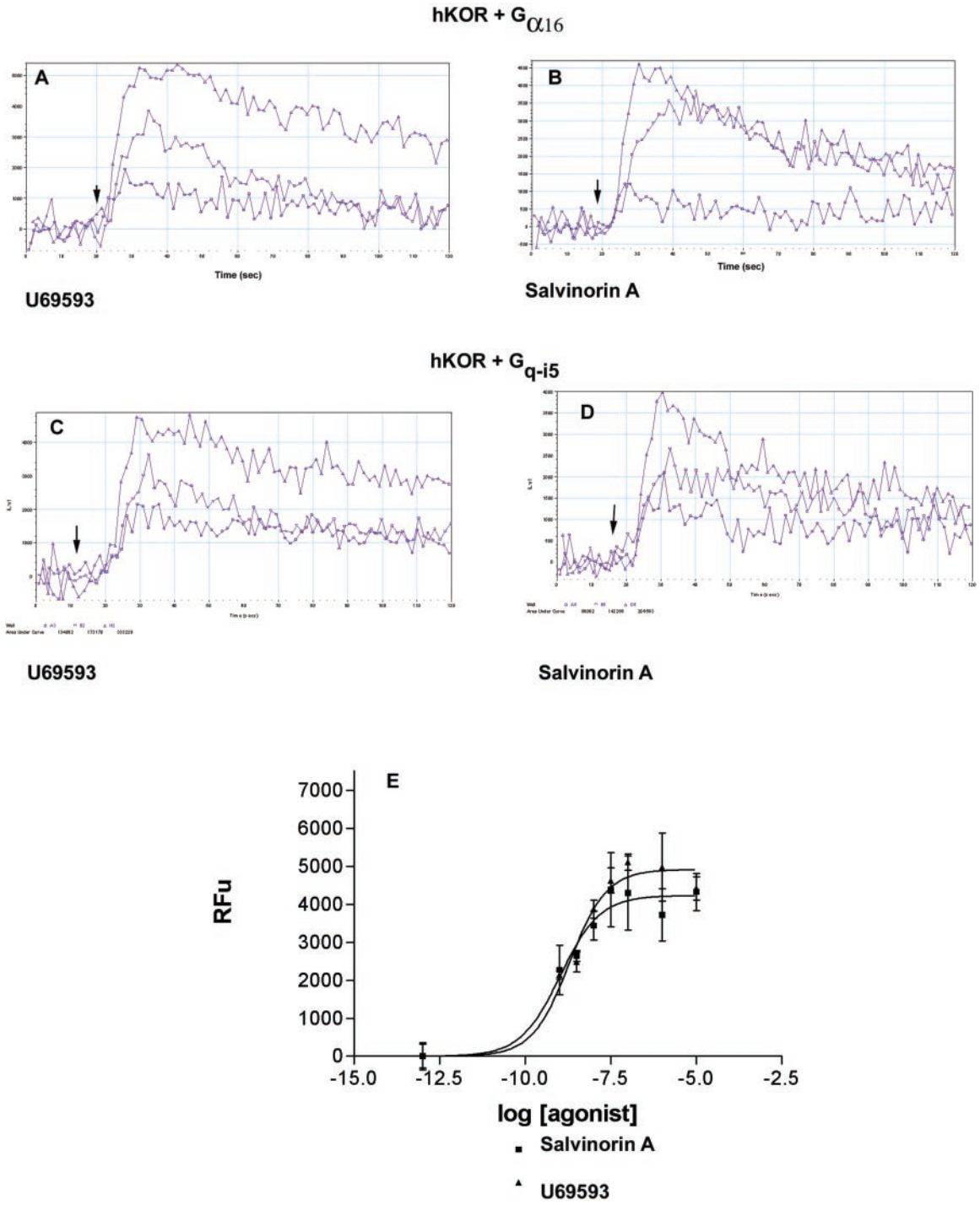

Fig. 2. Salvinorin A mobilizes intracellular Ca^{2+} when hKORs are cotransfected with the universal G protein G16 or the chimeric G protein. For these studies human embryonic kidney-293 cells were transfected with hKOR and either Gqi5 or G16 and the mobilization of intracellular calcium quantified as described previously (Rothman et al., 2004) using a 96-well FLEXSTATION. A and B, representative results with increasing doses of U69593 or salvinorin A (0, 10, and 100 nM), whereas hKORs were cotransfected with G16. C and D, results obtained when hKORs were cotransfected with Gq-i5. E, average for $n = 3$ separate experiments for dose-response studies to salvinorin A and U69593.

significant activity. One potential complication of the studies performed on recombinant, overexpressed receptors relates to the issue of receptor reserve. Thus, it is widely appreciated that overexpressing G proteins and/or receptors in heterologous expression systems leads to inaccurate estimates of agonist potencies and maximal responses (for review, see Kenakin, 1997). Accordingly, we also evaluated the agonist actions of salvinorin A and other compounds at KORs expressed in *Xenopus* oocytes.

KOR expressed in *Xenopus* oocytes activate intrinsic G proteins that then increase the conductance of coexpressed G protein-coupled inwardly rectifying potassium channels (GIRK and Kir3) (Henry et al., 1995). Injection of cRNAs coding for the mammalian receptor and channel has been

TABLE 3
Salvinorin A and salvinorinyl-2-propionate are agonist at hKOR-stimulated intracellular Ca^{2+} mobilization: comparison with reference compounds

Data represent mean ± S.D. of quadruplicate determinations for EC_{50} and E_{max} for mobilization of intracellular calcium.

Drug	EC_{50} in nM (pEC_{50} ± SD)	E_{max} (Relative to U69593)
U69593	13 (1.14 ± 0.2)	100
U50488	24 (1.39 ± 0.14)	102 ± 4
Salvinorin A	7 (0.84 ± 0.07)	104 ± 7
Dynorphin A	83 (1.92 ± 0.17)	107 ± 8
Salvinorinyl-2-propionate	17.3 (1.23 ± 0.18)	102 ± 8
Salvinorin B	No activity	No activity

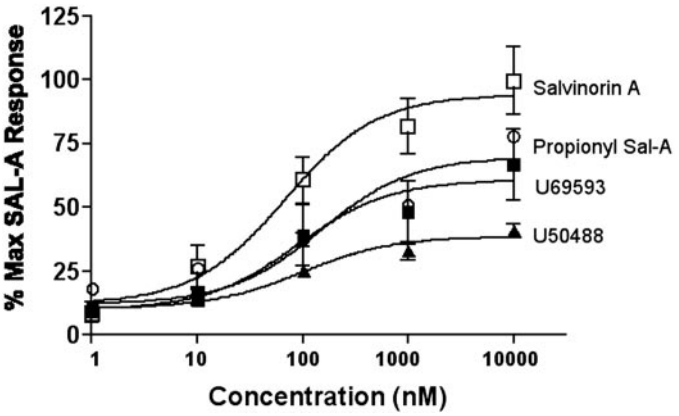

Fig. 4. Concentration-response curve of salvinorin A, and κ-agonists U69593 and U50488. Cumulatively higher concentrations of salvinorin A and the κ-agonists were applied to the bath. The agonist response at each concentration was normalized as a percentage of the maximal salvinorin A response. Each point represents the mean response measured in four to seven different oocytes.

demonstrated to faithfully reconstitute opioid signaling in oocytes equivalent to that observed in guinea pig substantia gelatinosa neurons (Grudt and Williams, 1993). In addition, by controlling the levels of receptor and channel expression, spare receptors can be avoided and the peak responses produced by different drugs can be a direct measure of agonist efficacy. The in vitro bioassay also eliminates pharmacokinetic barriers, and the electrophysiological recording of channel activation provides a rapid measure of receptor activation. In this study, we compared the relative activity of salvinorin A with three compounds having established κ-opioid receptor agonist activity. Salvinorin A was found to be more potent and have higher efficacy than either U50488 and U69593. The agonist efficacy of salvinorin A was not significantly different from dynorphin A(1-17), an endogenous neurotransmitter of the κ-opioid receptor (Chavkin et al., 1982).

Structure-activity relationship studies show that the KOR agonistic activity of salvinorin derivatives depend largely on the size and character of the substituent on the 2-ester moiety. Generally, the studied derivatives have either lower affinity for KOR than salvinorin A or are completely devoid of activity. The two active derivatives, the propionate and the heptanoate, demonstrate that as the alkyl chain is lengthened, KOR affinity diminishes. Interestingly however, chain length must not be the only factor, because the short-chain ethylcarbonate derivative is absent of activity.

The current results support the conclusion that just as morphine is a natural plant product able to activate the μ-opioid receptor, salvinorin A is a natural plant product able to activate the KOR. The strongly psychotomimetic actions of salvinorin A suggest that the dynorphin/κ-opioid system may have a role in the regulation of cognition and perception and support earlier proposals that some forms of schizophrenic hallucinations may be caused by hyperactive endogenous opioid systems (Gunne et al., 1977). Recent data implicating the KOR-dynorphinergic system in modulating stress and anxiety responses in rodents suggest that targeting KORs might also lead to novel antidepressant and anxiolytic medications. Salvinorin A, by virtue of its potency, efficacy, and selectivity as a KOR agonist will be an important tool for discovering the role that the KOR-dynorphinergic system has in health and disease.

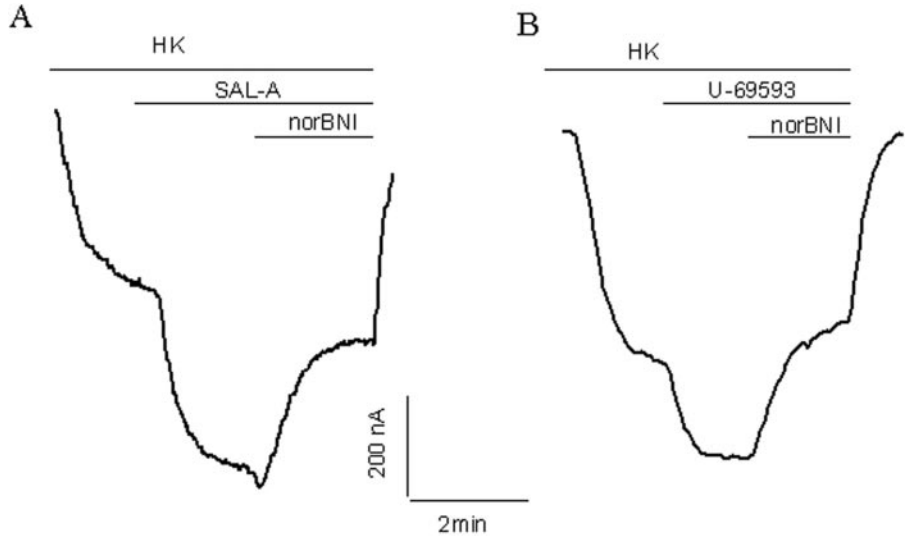

Fig. 3. Salvinorin A is a highly efficacious κ-receptor agonist. Representative traces showing the change in current during a typical experiment. A large inward current was apparent as the K^+ concentration was increased from 2 to 24 mM in normal oocyte saline buffer. Salvinorin A (1 μM) and U69593 (1 μM) in the buffer (24 mM K^+) further increased Kir3 currents, and the response was reversed by nor-BNI (100 nM), a κ-antagonist.

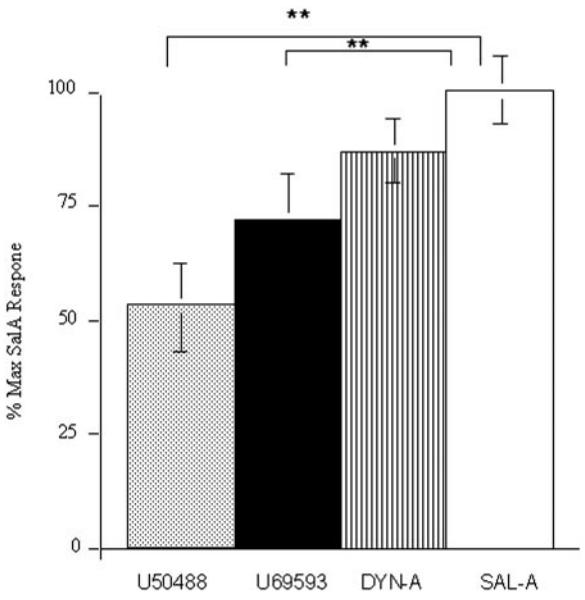

Fig. 5. Salvinorin A is more efficacious than U69593 and U50488 in κ-receptor-mediated activation of Kir3 currents. At saturating concentration, salvinorin A (10 μM) evoked a large Kir3 currents, which were significantly higher than the response evoked by U69593 (10 μM) or U50488 (10 μM). Data are mean ± S.E.M.; **, $p < 0.05$. Dynorphin A (10 μM) produced a response that was not significantly different from salvinorin A.

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The Absolute Stereochemistry of Salvinorins

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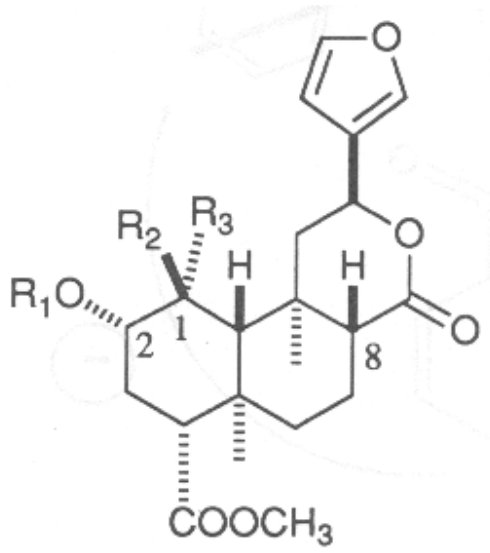
The absolute stereostructures of the hallucinogenic diterpenes Salvinorin A and B have been unambiguously determined by the use of the non-empirical exciton chirality circular dichroism method on their 1α,2α-diol dibenzoate derivative.

Recent investigations[1,2] of the hallucinogenic Mexican mint *Salvia divinorum*[3] have resulted in the isolation of the pharmacologically active diterpene salvinorin (divinorin) A (**1**) and its desacetyl analog salvinorin B (**2**). Extensive ^{1}H and ^{13}C NMR studies on these *trans*-clerodanes[1,2] and their derivatives,[2] as well as single-crystal X-ray analysis,[1,2] have led to the formulation of the structures of these compounds. The absolute chemistry of the salvinorins was postulated based on the observed negative n→π* Cotton effect of the 1-ketone around 295 nm in their circular dichroism (CD) spectra.[1,2] While this assignment had appeared to be corroborated by the n→π* Cotton effect of isofruticolone,[4] the ambiguous nature of the approach associated with this empirical CD method necessitated an independent, unequivocal verification of the absolute stereochemistry. In the following, we delineate the unambiguous assignment of the absolute stereochemistry of these physiologically important diterpenes through the use of the non-empirical exciton chirality CD method.[5]



1 $R_1 = Ac, R_2 = R_3 = O$
2 $R_1 = H, R_2 = R_3 = O$
3 $R_1 = R_2 = H, R_3 = OH$

In an effort to obtain a salvinorin derivative possessing an α-diol system which can be transformed into the dibenzoate ester required for the exciton chirality CD method, salvinorin A (**1**) or B (**2**) was treated with sodium borohydride in various protic solvents. The products having the 1α,2α-diol group were obtained in high yield. However, this reduction was accompanied by extensive isomerization at C-8. While mechanistic details for this unexpected observation remain to be established at this time, the isomerization at C-8 appears to be the result of the base-promoted clevage of the C-8/9 bond under the reaction conditions followed by the reclosure to provide the 8-epimer prior to the reduction of the 1-ketone. Furthermore, attempts to obtain the 1,2-dibenzoate derivative of the major reduction product **3** under various benzoylating conditions invariably produced only the 2-monobenzoate.

Since it was deemed desirable to remove possible interaction between the benzoate and the furan chromophores for the unambiguous CD analysis, salvinorin A (**1**) was reduced under cataytic hydrogenation conditions, providing the hexahydro derivative **4** (a 2:1 epimeric mixture at C-13) after esterification with diazomethane and desacetylation with KCN/MeOH.[6)] Interestingly, ester **4** was found to be relatively stable towards configurational isomerization at C-8. Thus, reduction of **4** with $NaBH_4$ in EtOH produced cleanly the *cis*-1α,2α-diol **5** in 81% yield. The benzoylation of the 1-α-hydroxyl group in

5, which is surrounded by the two 1,3-diaxially juxtaposed methyl groups, proved to be quite difficult under the standard benzoylation conditions. However, treatment of **5** with trimethyl orthobenzoate at 100°C in the presence of a catalytic amount of benzoic acid followed by acid-catalysed hydrolysis of the resulting 1,2-cyclic orthobenzoate provided the 1-monobenzoate derivative of **5**.[7,8] Benzoylation of this monobenzoate under standard conditions afforded the desired 1,2-dibenzoate **6**[9] in 95% yield. Alternatively, treatment of diol **5** with benzoyl triflouromethanesulfonate (BzOTf)[10] resulted in the direct formation of **6** in 50%yield.

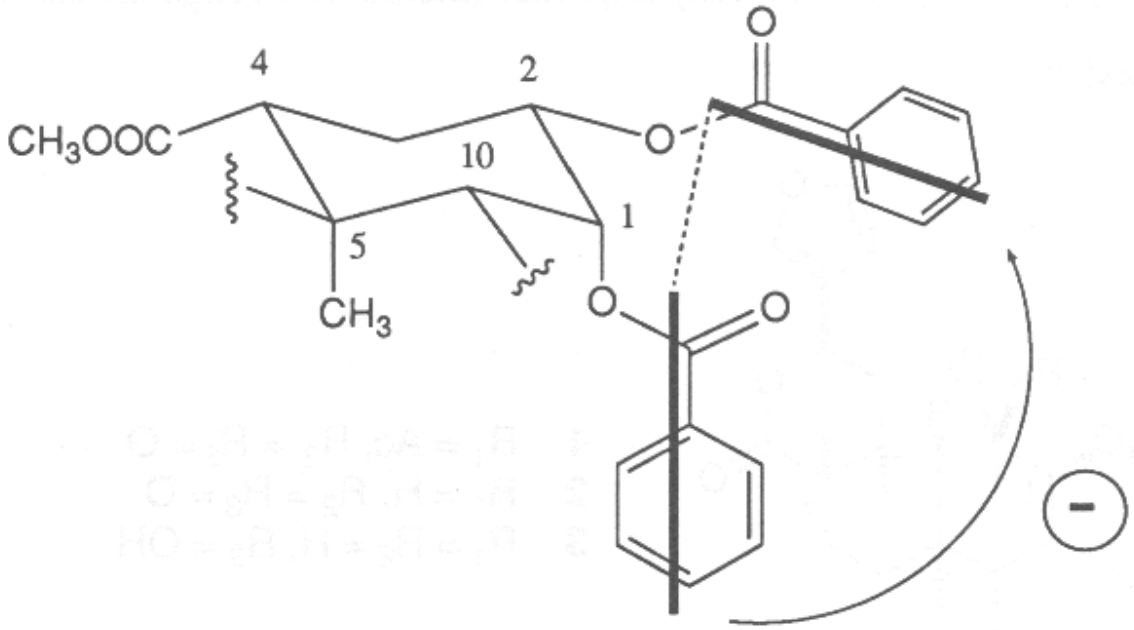
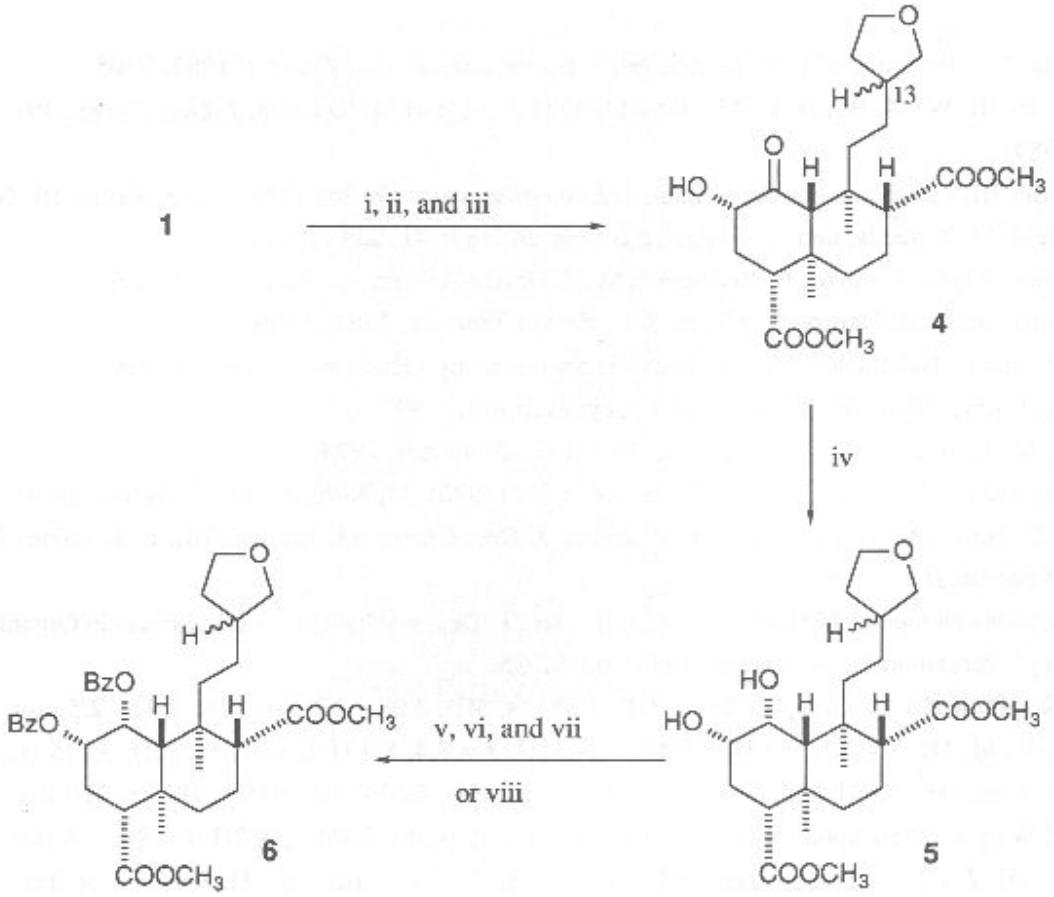


Fig. 1. The negative chirality between the two benzoate electric transition dipoles of the 1,2-dibenzoate derivative **6**.



Scheme 1. *Reagents and conditions*: i, H_2, 5% Pd/C/MeOH, 14 h; ii, CH_2N_2/MeOH, 0°C, 2 h; iii, KCN (3.0 equiv.)/MeOH, reflux, 15 min [74% yield for **1** → **4**]; iv, $NaBH_4$ (5.0 molar equiv.)/abs. EtOH, 0°C → room temperature, 12 h (81%); v, $PhC(OME)_3$ (excess), PhCOOH (catalytic), 100°C, 1 h; vi, THF/water/AcOH (15/5/1), conc. HCl (2 drops) (65% yield for v and vi); vii, BzCl (excess)/pyridine, room temperature, 2 h (95%); viii, BzOTf (5 equiv.), pyridine (7.5 equiv)/CH_2Cl_2, -78°C → room temperature, 1 h at room temperature (50%).

The CD spectrum of the 1,2-dibenzoate **6** in 9:1 MeOH/dioxane showed a pair of typical exciton-split Cotton effects with opposite signs centred upon the UV absorption (227 nm) of the benzoate chromophore: $\Delta\varepsilon_{235.5}$ -15.9 and $\Delta\varepsilon_{221.5}$ +6.66. The negative longer

wavelength Cotton effect clearly defines the negative chirality between the two electric transition dipoles of the benzoate chromophores assignable to the long axis $\pi \rightarrow \pi^*$ transitions (Fig. 1),[5] thus unequivocally assigning the absolute stereostructures of salvinorin A and B as given in **1** and **2**, respectively.

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9. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.121 (s, 3H), 1.544 (s, 3H), 2.08-2.23 (m, 2H), 2.41-2.56 (m, 3H), 3.419 (dd, 1H, *J* = 7.6, 7.6 Hz), 3.445* (dd, 1H, *J* = 8.4, 8.4 Hz), 3.647 (s, 3H), 3.715 (s, 3H), 3.75-4.03 (m, 3H), 5.051 (ddd, 1H, *J* = 11.8, 4.5, 3.5 Hz), 6.024* (m, 1H), 6.047 (m, 1H; the observed $W_{1/2}$ = 3.8 Hz upon irradiation of the 5.051 ppm peak), 7.259 (dd, 2H, *J* = 8.0, 7.6 Hz), 7.457 (tt, 1H, *J* = 7.6, 1.2 Hz), 7.494 (dd, 2H, *J* = 8.1, 7.7 Hz), 7.620 (tt, 1H, *J* = 7.7, 1.4 Hz), 7.756 (dd, 2H, *J* = 8.0, 1.2 Hz), and 8.043 ppm (dd, 2H, *J* = 8.1, 1.4 Hz). The peaks with asterisks indicate those of the spectroscopically resolved minor C-13 epimer.

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Ethnopharmacognosy and Human Pharmacology of *Salvia divinorum* and Salvinorin A

Jonathan Ott

Zusammenfassung: Der Autor gibt einen historischen Überblick zu Gebrauch und Forschungsgeschichte der Wahrsagesalbei *(Salvia divinorum).* Es werden der traditionelle Gebrauch bei Schamanen der Mazateken in Oaxaca/Mexiko sowie der nichttraditionelle,moderne Gebrauch verschiedener Zubereitungsformen von nordamerikanischen »Keller-Schamanen« vorgestellt und ausftihrlich diskutiert. Der Frage nach der botanische Identitiit des »verlorenen« aztekischen Entheogens *pipiltzintzintli* wird nachgegangen. Schließlich stellt der Autor seine Selbstversuche mit der sogenannten »Heffter-Technik« vor.

Abstract: After a thorough review of the limited ethnographic data on shamanic use of the entheogenic mint *Salvia divinorum* by the Mazatec Indians of the Sierra Madre Oriental of the Mexican state of Oaxaca, with special emphasis on pharmacognostical aspects, the author details the phytochemical studies which led to the isolation of the novel diterpene salvinorin A in 1982-1984. *Lingering* doubts as to the visionary properties of this compound were laid to rest a decade later, when 'basement shamans' in the United States isolated and tested the compound in psychonautic bioassays. A tabular summary of 15 reports involving at least 60 trials of the novel drug by human volunteers is presented; documenting activity of infusions of *Salvia divinorum* leaves in water [the traditional method of ingestion], of the fresh leaves chewed, whether subsequently swallowed or retained in the mouth as a quid; and of the dried leaves smoked. Pharmacological activity of salvinorin A in human volunteers is likewise discussed, both for inhalation of the vaporized compound and sublingual application of 1 % solutions in acetone or dmso; including original research here reported for the first time. Extremely low thresholds for psychoactivity of salvinorin A [100-250 mcg sublingual; 200-500 mcg vaporized and inhaled] show this compound to be the most potent natural product entheogen known; some 10 times the potency of psilocybine from mushrooms likewise used as shamanic inebriants by the Mazatec and other Mexican Indians, and more than 1000 times the potency of the prototypical entheogen mescaline, from the peyotl cactus [*Lophophora williamsii*] used as a visionary drug by the Huichol, Tarahumara and other indigenous peoples of northern Mexico. Speculations regarding the status of *Salvia divinorum* as a cultigen are discussed, as is R. Gordon Wasson's conjecture that this plant represents the lost Aztec entheogen pipiltzintzintli. An exhaustive bibliography of more

than 70 references reviews the ethnographic, chemical and pharmacological literature on this intriguing shamanic inebriant.

Keywords: Mazatec Indians, Aztecs, Mesoamerica, entheogens, Pipiltzintzinli, Heffter Technique

The Mexican divinatory mint, *Salvia divinorum* Epling et Játiva, is one of the most obscure and mysterious of all shamanic inebriants. Unlike its more famous Mexican relatives, the *péyotl* cactus *Lophophora williamsii* (Lemaire) Coulter, *teonanácatl,* the psilocybian mushrooms and *ololiuhqui,* seeds of the morning glory *Turbina corymbosa* (L.) Rafinesque, this plant largely or completely escaped the notice of the 16th and 17th century Spanish friars and the opprobrium of the Holy Office of the Inquisition. Indeed, it was not even mentioned in the scientific literature until 1939 (Johnson 1939), was not described botanically until 1962 (Epling & Játiva-M. 1962) and it wasn't until 1993 that its active principle was finally identified (Siebert 1994). Actually, this active principle, salvinorin A, was first isolated in 1982, in the course of a systematic chemical search for novel terpenoid compounds in the genus *Salvia* (Ortega et al. 1982). Although the Valdés group, searching for the psychoactive principle of this drug, independently isolated the same compound two years later (giving it the synonym divinorin A), an imprecise animal assay was employed (the so-called 'Hall's open-field' bioassay in mice) (Valdés et al. 1984). Even 'though members of the Valdés group had ingested *Salvia divinorum* leaves in a traditional shamanic context in Mexico (Díaz 1975,1979; Valdés et al. 1983), they did not follow their research through to the definitive test of salvinorin A in psychonautic bioassays, the only valid proof this compound represented the visionary active principle of the leaves. Only when non-professional, countercultural 'basement shamans' commenced experimentation with the crude drug a decade later, was the conclusive 'Heffter Technique' employed, and human self-experiments showed beyond doubt that salvinorin A is the main visionary principle of *Salvia divinorum.*

The pioneering Swedish anthropologist Jean Bassett Johnson, first scientist to observe divinatory use of Mexican entheogenic mushrooms in the summer of 1938, in the Mazatec village of Huautla de Jiménez, also mentioned in passing that:

> "In addition to the mushrooms, some people use a seed called *'Semilla de la Virgen,'* others use *'Hierba María.'* ... the Zapotec use a plant called *'bador,* the little children,' which is administered in the same way as *yerba María* by the Mazatec. The leaf is beaten well, and a tea is made thereof ..."

referring presciently both to the entheogenic morning glory seeds (known as *badoh* in Zapotec or *semillas de la virgen* in Spanish) (Ott 1993) and *Salvia divinorum* (Johnson 1939). Six years later the Austrian physician Bias Pablo Reko, great pioneer in the field of Mexican ethnopharmacognosy (not to be confused with his cousin Victor Reko, a *farceur* who gained prominence in the German-speaking world by appropriating the fruits of his cousin's work in an unscientific popular book, *Magische Gifte)*, mentioned the use, by the Mazatec and neighboring Cuicatec Indians of Oaxaca, of an *hoja de la adivinación* (divinatory leaf), in all probability *S. divinorum* (Reko 1945). Yet another clue was provided in 1952 by the great Mexican anthropologist Roberto J. Weitlaner, also an

Austrian, when he described the therapeutic and divinatory use of an aqueous potion made by 'rubbing the leaves (50-100) in water' of a *Yerba de María* (Weitlaner 1952):

> "otra yerba que en su pueblo se llama Yerba de María ... se utilizan las hojas, poniendolas en agua. Primero se fro tan entre las manos ... EI enfermo bebe el agua en que se han frotado las hojas ... Esperan un cuarto de hora el efecto de la droga y el mismo e_fermo empieza a decir la cIase de enfermedad que padece ... Cuando amanece el curandero bafia al enfermo con agua de la misma que torno, y con esto queda curado el enfermo. (another herb known in his village as Herb of Mary... the leaves are used, putting them in water. First one rubs them between the hands ... The patient drinks the water in which the leaves have been rubbed ... They await the effect of the drug for a quarter of an hour and the patient himself begins to state what type of sickness he suffers ... At dawn the *curandero* bathes the patient with the same water he drank, and thus the patient is cured."

However, it was the diligent work of the pioneering ethnomycologist and entheogenic ethnopharmacognosist R. Gordon Wasson which finally led to the collection of botanical voucher specimens of this plant in October 1962. Wasson was also the first scientist on record to have ingested the divinatory leaves, which his botanical collaborators Carl Epling and Carlos D. Játiva-M. subsequently identified as a new species, *Salvia divinorum* (Epling & Játiva-M. 1962; Wasson 1962). Just as important as the identification of the plant and documentation of its effects was Wasson's collection of live material, which then began to be cultivated in the United States—it was from this so-called 'Wasson clone' that salvinorin A was isolated in Los Angeles in 1993, at last allowing testing of this compound in human beings (Siebert 1994).

Wasson first ingested the divinatory leaves in Ayautla on 12 July 1961, when he was given a potion of the diluted, handsqueezed juice of 34 pairs of leaves, and compared the resulting effect to that of the psilocybian mushrooms:

> "The effect of the leaves came sooner than would have been the case with the mushrooms, was less sweeping, and lasted a shorter time. There was not the slightest doubt about the effect, but it did not go beyond the initial effect of the mushrooms—dancing colors in elaborate, three-dimensional designs."

Wasson also mentioned his ingestion of the juice of merely five pairs of leaves in San José Tenango on 9 October 1962, on which occasion Anita Hofmann, wife of Albert Hofmann, ingested the juice of only three pairs:

> "We both felt the effects, which were as I described them in the ceremony in Ayautla the year before."

Two days later in Huautla de Jiménez, while María Sabina was celebrating a mushroom *velada* with pills of *Indocybin*® or synthetic psilocybine, Albert Hofmann likewise ingested the infused juice of five pairs of *S. divinorum* leaves (Hofmann 1979, 1990), but unlike his wife and Gordon Wasson, he experienced only:

> "a state of mental sensitivity and intense experience, which, however, was not accompanied by hallucinations."

In his pioneering paper on *Salvia divinorum* (Wasson 1962), and an important sequel the following year, summarizing ethnobotanical data on the major Mexican entheogenic plants (Wasson 1963), Wasson detailed what he had been able to learn about the

divinatory leaves. They seemed to be used only by the Mazatecs, who called them *ska Pastora* or the equivalent in Spanish, *hojas de la Pastora* or *hojas de María Pastora* ('leaves of the Shepherdess' or 'leaves of Mary Shepherdess'). This odd name has not received the comment it is due. The interpolation of *María* into the name suggests the Catholic influence which has corrupted Mexican shamanism, but the Biblical Mary was no shepherdess, nor does any such woman figure in Catholic iconography. More importantly, however, the Mazatecs would not have seen sheep until after the arrival of Europeans to Mexico in the sixteenth century. This name is clearly a modernism, and it is more than surprising that an important shamanic inebriant would lack an indigenous name, for 'leaves of Mary Shepherdess' can in no way be considered an indigenous name, for a people whose pre-Columbian ancestors never set eyes on a sheep! It is even conceivable that *Salvia divinorum* use is a post-Conquest introduction to the Sierra Mazateca. We will return to this point below.

Wasson described two methods of ingestion of *Salvia divinorum* leaves: either by making a stack of leaves in pairs face-to-face, which are then simply eaten ("It is customary for the Indians to consume the leaves by nibbling at the dose with their incisor teeth."); or in the form of their juice, or rather a sort of aqueous suspension of the leaves in cold water. This latter was precisely the method documented by Weitlaner. Thus was prepared Wasson's first dose of the leaves in Ayautla:

> "Augustina squeezed the leaves with her hands and collected the juice in a glass. This was certainly an inefficient method. Some water was added. I drank the dark fluid, about half a glass full, the result of squeezing 34 pairs ..."

As for the dose of five pairs of leaves prepared in San José Tenango the following year for Wasson and the three pairs for Anita Hofmann, these were:

> "ground ... on her *metate,* after passing them through the smoke of *copal,* and she did a thorough job of it. Water is added to the mass that comes off the metate, the whole is put through a strainer, and then we drank the liquor."

Wasson also mentioned the curious *datum* that the Mazatecs regarded *Salvia divinorum* to be the most important member of a 'family' (all, botanically speaking, indeed members of the same family, Labiatae), being *la hembra,* 'the female,' whereas *el macho* or 'the male' was *Coleus pumilus* Blanco, and *el nene,* 'the child,' or *el ajihado,* 'the godson,' was *Coleus blumei* Bentham. This is more than strange, given the fact that both species of *Coleus* are post-conquest introductions to Mexico (Schultes 1967), and their juxtaposition with *Salvia divinorum* in the minds of the Mazatecs might be seen as reinforcing the suspicion that their use of the 'leaves of Mary Shepherdess' too is a post-conquest innovation. Unfortunately, we have no firm evidence for the psychoactivity of either species of *Coleus.* Wasson "tentatively" suggested that *Salvia divinorum* might represent the unidentified pre-conquest Nahua entheogen *pipiltzintzintli,* (or *pepetichinque)* mentioned by 17th century friar Agustin de Vetancurt and in the annals of the Inquisition, as an herb taken in water for divination or applied in water as a poultice (recall Weitlaner's report that apart from drinking the infusion of *Yerba de María,* the patient was bathed in it) (Aguirre Beltran 1963; Garza 1990; Vetancurt 1698; Wasson 1963). It has also been suggested that *Salvia divinorum* is represented in the head dress of

a deity depicted in the Mayan *Dresden Codex* (Emboden 1983). In her 1977 biography, Mazatec shaman María Sabina (one of Wasson's primary informants) noted that:

> "Si tengo a un enfermo en el tiempo en que no se consiguen hongos, recurro alas hojas de la Pastora. Molido y tornado, trabajan como los *ninos*. Desde luego, la Pastora no tiene la fuerza suficiente. (If I have a patient during the season in which it is impossible to procure mushrooms, I have recourse to the leaves of the Shepherdess. Crushed and ingested. they work like the *children* (the mushrooms). Of course, the Shepherdess does not possess enough strength." (Estrada 1977)

Three years earlier, in a monumental transcription, transliteration and translation of an entire mushroomic curing ceremony with Sabina, Wasson had puzzled over María's repeated mentions of so-called 'aquatic leaves' which cured when rubbed on the patients' body (Wasson et al. 1974). Given Weitlaner's report of bathing patients in the *Salvia divinorum* infusion, most decidedly a cutaneous application of 'aquatic leaves' (as we will see, a decade later use of the leaf residue of *S. divinorum* infusions as a poultice was also reported), and Vetancurt's report of similar use of *pipiltzintzintli,* it seems probable that here María was speaking figuratively of external use of *Salvia divinorum,* a plant which is also 'aquatic' in it ravine habitat (Epling & Játiva-M. 1962).

This effectively summarizes our primary ethnographic data on *Salvia divinorum,* and Epling and Játiva's terse one-and-a-half-page paper, and Wasson's concise seven-page paper certainly provided little detail. It is thus surprising to note the relatively strong impact the leaves of the Shepherdess began to have on the literature. No fewer than five different color paintings of *Salvia divinorum* have been published (Emboden 1972; Foster 1984; Schultes 1976; Schultes & Hofmann 1979; Schultes & Smith 1980), along with two different botanical illustrations (Mayer 1977; Schultes 1967; Schultes & Hofmann 1973), two black-and-white photographs of the whole plant (Díaz 1975; Wasson 1963), and color and black-and-white photographs showing the use of a *metate* to prepare infusions of *Salvia* leaves (Riedlinger 1990; Wasson 1963)! Three of these paintings (Emboden 1972; Schultes 1976; Schultes & Smith 1980), one by Frances Runyan, two by Harvard botanical artist Elmer W. Smith, unfortunately misrepresented the corollas of *Salvia divinorum* as being purple, not white (in the botanical description Epling and Játiva had misdescribed the calyx color as "cyaneorum"; in the 1979 revised edition of Emboden 1972; the erroneous painting was replaced with a color photograph of the flowering plant, and Emboden amended the botanical description of the flowers). Fortunately this evident scientific interest led to renewed and more detailed studies of the mysterious entheogen. The Mexican psychiatrist José Luis Díaz began to study *Salvia divinorum* in the Sierra Mazateca in summer 1973, and in his preliminary paper he described the use of doses of 25 to 50 pairs of leaves, prepared by a manual technique similar to that previously described by Weitlaner (Díaz 1975):

> "toma una jicara con agua y sabre ella machaca vigorosamente el manojo de hojas con sus manes hasta que se extrae toda 'la sangre de la hojita.' El bagazo se desecha y el bebedizo resulta un liquido verde espumoso y en extrema amargo. (she takes a jar of water and using her hands vigorously mashes the bunch of leaves above it until all of the 'blood of the little leaf' is extracted. The bagasse is set aside and the resulting potion is an extremely bitter and frothy green liquid."

Díaz chronicled six personal experiences with the potion, of a total of 12 by members of his group, mentioning that "my perception of the effects has in general increased with

experience." Nevertheless, Díaz described quite mild visual effects (in some cases none at all) "far from being hallucinations," with the peak effects lasting only ten minutes and disappearing within a half-hour of ingestion. Díaz also described inconclusive chemical studies, stating there were: "various alkaloids in *Salvia divinorum,* two of which are apparently psychoactive." Díaz reported crude pharmacological experiments with "alkaline extracts" of the plant in cats (using the fractions which would correspond to defatted, acidic-water-soluble, basic-water-insoluble, alkaloidal constituents in a standard solvent extraction of alkaloids) commenting that effects were "notably similar to those produced by hallucinogens of the LSD type," which were, however, of much shorter duration, lasting at most a half-hour. Díaz also mentioned the inconsistent nature of the observed effects, which he ascribed to varying potency of the starting material or instability of the active agents (Díaz 1975,1977).

Albert Hofmann, who together with Gordon Wasson collected the first botanical voucher specimens of *Salvia divinorum* in October 1962, also made reference to this presumed instability of the active principles of *Salvia divinorum,* inasmuch as he had returned to Switzerland with juice of *Salvia divinorum* "preserved with alcohol" which "proved in self-experiments to be no longer active," thus depriving Hofmann and his coworkers of the Heffter Technique bioassay needed to guide the experimental isolation of the active principles (Hofmann 1979, 1990; Ott 1994, 1995a). It has been incorrectly stated in the literature that Hofmann made unsuccessful chemical attempts to isolate the active principle of *Salvia divinorum* (Valdés 1994b; Valdés et al. 1987a), when in reality he abandoned plans to study juice of the plant chemically, when it proved in self-experiments to be inactive. It is worth noting that Hofmann had simply expressed the juice of the leaves and diluted this with alcohol, rather than preparing the aqueous infusion of the 'rubbed' leaves described by Weitlaner, Díaz and Wasson.

Thus matters stood until 1979 and 1980, when Leander J. Valdés III began to collaborate with Díaz, making the isolation of novel compounds from *Salvia divinorum* his thesis project at the University of Michigan. Valdés described in great detail two shamanic healing sessions with Mazatec *curandero* Don Alejandro on 18 August 1979 and 6 March 1980. On both occasions Díaz and Valdés ingested infusions of *Salvia divinorum*--only in the first session did Don Alejandro likewise ingest the drug. Valdés described the divinatory dose of the leaves as being "from 20 (about 50 g) to 80 (about 200 g) or more pairs of fresh leaves to induce visions" (noting also A. Gomez Pompa's notations on herbarium sheets, to the effect that 8-12 pairs of leaves went into a dose); while in the 18 August session he received a "beginner's dose" made from 20 pairs and Díaz and Don Alejandro from 50 pairs; in the second session Díaz received a dose made from 60 pairs, Valdés from 50. Valdés mentioned that "only fresh foliage will serve for divination," that being a primary use for the leaves, which were also employed in shamanic training, and in lower doses as specific medicines for various diseases (Valdés et al. 1983). Valdés stressed the necessity of using only fresh leaves, noting in a second paper "it purportedly loses psychotropic activity on drying" (Valdés et al. 1987a). He also mentioned the existence of a prescribed *dieta* or ritual diet of 16 days, then reduced to only 4 days after the initial dose. Such a diet is also associated with the shamanic use of psilocybian mushrooms among the Mazatecs (Wasson & Wasson 1957), and is commonly prescribed with shamanic use of *ayahuasca* in Amazonia (Ott 1994) and with other shamanic inebriants. As in the reports of Weitlaner, Díaz and Wasson, Don

Alejandro apportioned pairs of the leaves which were crushed manually (Valdés et al. 1983):

> "into a small enameled bowl partially filled with water. As more foliage was squeezed and added, the liquid turned dark green ... (and) was poured through a sieve into a glass which was topped off with water."

Supposedly the leaves could be kept fresh for up to a week by wrapping them in leaves of *Xanthosoma robustum* Schoff, but the infusion would only last for a day. Whereas the leaf residue was usually left in a remote place, it was sometimes applied as a poultice to the head of a patient, again harking back to Vetancurt's 17th century description of *pipiltzintzintli* (Garza 1990). Díaz described the commencement of subtle visions 15 minutes after ingesting the infusion of 50 pairs of leaves on 18 August (his seventh experience), which became more intense over the next 15 minutes. Valdés also described visions, and a sensation of flying, 45 minutes after ingesting his infusion of 20 pairs of leaves. Both Díaz and Valdés described visions during the first hour of the session of 6 March, which was cut short at the 50-minute point, owing to distracting noises. Even 2.5 hours after ingestion, having returned to his hotel and extinguished the light, Valdés experienced more visions, and the sensation of the perceived reality of:

> "standing in a bizarre, colored landscape talking to a man who was either shaking or holding on to his hand. Next to them was something that resembled the skeleton of a giant (sic) stick-model airplane made from rainbow colored inner tubing. The 'reality' of what he was seeing amazed him." (Valdés et al. 1983)

Valdés later noted "It was an amazing hallucination, as I truly believed I was in the meadow. It was not like a dream." (Valdés 1994b), and such vivid visions of alien space or geometry are a hallmark of the effects of *Salvia divinorum* (Blosser 1991-1993). Both Díaz and Valdés experienced physical effects as well as visions, consisting of incoordination, dizziness and slurred speech. In contrast to Wasson's report that the leaf infusion "did not go beyond the initial effect of the (psilocybian) mushrooms," Valdés stressed "the *Salvia* infusion will induce powerful visions under the appropriate conditions" of silence and darkness.

As mentioned above, Valdés went on to isolate two novel *trans*-neoc1erodane terpenoid compounds from the leaves, which he named divinorins A and B (Valdés et al. 1984), only to discover that he had been 'scooped' by the group of Alfredo Ortega in Mexico, which had already isolated the more important of these compounds, giving it the name salvinorin (making salvinorin A and B the appropriate designations for the compounds) (Ortega et al. 1982). The Ortega group was not studying ethnopharmacognosy *per se,* but rather studying terpenoid chemistry in *Salvia* species, and they conducted no pharmacological tests of the novel compound. Valdés' group, on the other hand, was actively seeking the visionary principle of the plant, using as bioassay not the indicated Heffter Technique, but "a modification of Hall's open field" in mice. This involved administering fractions of the plant to mice, then observing their behavior in a 90 cm circle divided into squares, that is, counting the number of squares entered, time spent immobile, and rearings onto hind legs. They concluded that salvinorin A was the visionary principle of the plant, as it reduced all three measures of activity in the

mice, much as *Salvia divinorum* did in human beings ('though Valdés had not documented his nor Díaz's behavior in the open field, nor described either rearing up on his hind legs!). Furthermore, salvinorin A was said to have a sedative effect on the mice (while salvinorin B, its desacetyl congener, was inactive in this assay), and Valdés later published the details that all the following compounds provoked the same effect in the mouse bioassay as salvinorin A: mescaline, secobarbital, an ether extract of *Cannabis sativa* L. and another labiate terpenoid compound, the hypotensive forskolin or colforsin (Valdés et al. 1987a). Later, in a subsequent paper, Valdés qualified this, stating:

> "further testing ... has allowed a different interpretation ... amphetamine stimulated the mice; secobarbital, forskolin and the cannabis extract had strong sedating effects ... Mescaline, salvinorin A, and isosalvinorin A—the 8-epimer of salvinorin A—interrupted (decreased) animal activity without an accompanying true sedation ..."

and noting the activity of salvinorin A was qualitatively and quantitatively similar to that of mescaline (Valdés 1994b)! The fact that pharmacologically-disparate compounds like the potent sedative secobarbital and the powerful stimulant mescaline gave similar results in the bioassay, should have alerted the Valdés group to its lack of specificity, but they inexplicably neglected to employ psychonautic bioassays which would have left no doubts about the activity of the salvinorins. Valdés' group also mentioned the existence of "at least two more terpenoids" in their extracts, and noted that the terpene-enriched crude fraction of the leaves was "substantially stronger" than its equivalent of pure salvinorin A, and Valdés later reported his isolation from the leaves of the ant-repellent loliolide, of unknown pharmacology and previously found in various plants, including *Lolium perenne* L. (Valdés 1986). In seeming refutation of the Mazatec belief that the *dried* leaves are inactive, both the Ortega and Valdés groups isolated salvinorin A from dried leaves, and the latter group reported a yield of 0.18 % salvinorin A in dried leaves; corresponding to 0.022 % on a fresh weight basis. Neither group published a synthesis of salvinorin A (or B), but both derived the same structure from X-ray crystallography (it is unusual for this procedure to be carried out twice for the same compound), and the group of M. Koreeda subsequently worked out the absolute stereochemistry of salvinorins A and B (Koreeda et al. 1990). Valdés' group was unable to confirm the report of alkaloids in *Salvia divinorum* by Díaz, noting:

> "extensive work in our laboratory has shown that the pharmacologically active extracts from *S. divinorum* do not contain alkaloids, nor were we able to isolate any alkaloids from the plant itself." (Valdés et al. 1984)

Díaz's conclusions are generally regarded to have been premature, and it is an open question how (presumably) alkaloid-enriched extracts of the leaves were pharmacologically active in cats—it is my opinion that Díaz's bioassay itself was at fault.

Salvia divinorum (Photograph: Jonathan Ott)

Having written his thesis on the isolation of salvinorins from *Salvia divinorum* to get his PhD., Valdés concluded his research on the plant with some cultivation experiments in Ann Arbor, Michigan; outdoors in summer and in greenhouses the rest of the year. Manual cross-pollination of the 'Wasson clone' and a strain collected by Valdés resulted in 4 of 14 setting seed (28%), but the seed was accidentally killed by overheating the growth chamber before viability could be assessed (Valdés et al. 1987a). At this point Valdés' scientific research with *Salvia divinorum* was temporarily suspended, leaving the question of the active principle unresolved. Although Valdés' group suggested salvinorin A was the visionary principle (in their 1987 paper, Valdés et al. expressed reservations: "if salvinorin A and the new compounds we isolated ... prove to display hallucinogenic activity in humans"), the gross lack of discrimination of their bioassay left room for

doubt, and the simple expedient of testing the novel compound in a human researcher was inexplicably foregone.

The next chapter in the scientific biography was to be written by 'basement shamans' of the United States' 'counterculture.' As early as 1984, *Salvia divinorum,* baptized as 'diviner's sage' (Heffern 1974) or 'sage of the seers,' was profiled in a latter-day herbal (Foster 1984) which was recently reprinted. This book gave a concise summary of ethnographic data on the plant, described its cultivation, and mentioned the important *datum* that live specimens could be purchased from a California seed company identified in an appendix. Foster described his ingestion of 20 leaves:

> "leaving me with an upset stomach, a dry, acid mouth, and a great respect for Mazatecs who can work their way through a hundred! For me the leaves produced hardly noticeable effects. Craig Dremmond (sic) suggests that plants cultivated outside of Oaxaca may not develop the active constituents, and I predict that *Salvia divinorum* will never become a popular subculture euphoric."

This comment, and María Sabina's dismissal of the leaves as feeble compared to her preferred entheogenic ally *teonanacatl* (María's biography was translated into English in 1981, noting "Of course the Shepherdess doesn't have as much strength.") (Estrada 1977), have seemingly informed modem consciousness of this little-known entheogen, which acquired a reputation as being weak and second-rate (tacitly assumed of any plant our governments have not deigned to prohibit). Reviewing entheogens in a widely-read anthology, botanical expert Richard Evans Schultes commented (Schultes 1972):

> "In Oaxaca, *Salvia divinorum* seems to be utilized only when supplies of the mushrooms and morning-glory seeds are short"

Another more recent source echoed this theme of surrogate or second-rate entheogen (Rätsch 1988):

> "Mazatec shamans use its *(S. divinorum's)* leaves when they are unable to obtain magic mushrooms (Teonanacatl)."

Nevertheless, as early as 1973 *Salvia divinorum* was included in a popular booklet on *Growing the Hallucinogens* (Grubber 1973) and live plants continued to be available commercially, becoming a mainstay of the mail-order plant and seed companies dedicated to shamanic inebriants, which began to appear in the nineties, and whose customers became avid collectors and cultivators of such exotica. There even arose on-line computer bulletin board systems (b.b.s.) dedicated to shamanic inebriants and other psychoactive drugs, such as *alt.drugs, aft.drugs.psychedelics, alt.psychoactives* and myriad others, where 'basement shamans' could compare horticultural and other pharmacognostical notes. In 1992, one such entheogen *aficionado,* Jim Dekorne, started a newsletter, *The Entheogen Review,* in which readers could share experiences with novel and largely unknown drugs like *Salvia divinorum,* and report innovations in their cultivation, preparation and use.

I first encountered *Salvia divinorum* in 1975, when I moved to Mexico to collaborate with the Díaz group. I observed that young Mexican users of *Cannabis* and entheogenic mushrooms, who were wont to engage in mushroomic tourism to Huautla de Jiménez to obtain psilocybian mushrooms, which had become articles of the tourist trade there (Ott

1975), would return to Mexico City with dried leaves of *Salvia divinorum*, which they would smoke in 'joints,' like marijuana. I verified that the dried material was, in fact, active and effective when smoked, in contrast to the Mazatec belief that drying the leaves destroyed their potency. This observation was first reported in the literature by Díaz, in his first paper dealing with *ska Pastora* (Díaz 1975). Smoking dried *Salvia divinorum* leaves surprisingly became the preferred mode of ingestion among certain users in the United States (Pendell 1995). By the summer of 1993, *Salvia aficionados* in California had discovered that by far the most potent means of ingesting the fresh leaves was the so-called 'quid method,' chewing the leaves well and retaining the leaf mass and juice in the cheek, in the manner in which *coca (Erythroxylum coca* LAM.) is typically chewed, swallowing neither the leaves nor their juice. Valdés, with whom the 'basement shamans' communicated this finding, later mistakenly reported that the Mazatecs so use the leaves:

> "Some Mazatecs, as well as nonnative experimenters, chew a cocalike quid of the fresh leaves that induces strong and persistent visions ... Mazatec informants made a quid of four to five pairs ..." (Valdés 1994b)

In fact, this method was discovered by non-professional researchers in California, again besting the Mazatecs, who failed to discover this most effective method of ingestion, just as they failed to discover the activity of dried leaves or their activity when smoked. Finally, in the summer of 1993, these same 'basement shamans' succeeded in isolating a salvinorin A-enriched crude precipitate (which I verified shortly after to be roughly 50% pure) from organic solvent extracts of the dried leaves (the procedure was shown to me, and it involved the simplest possible kitchen chemistry, which could be executed in less than an hour), and demonstrated by smoking this precipitate on tinfoil or in glass pipes that it was active at doses of around 1 mg, and did indeed contain the visionary principle of the leaves. After Valdés provided a sample of authentic salvinorin A, it was irrefragably shown that the precipitate was impure salvinorin A, thus proving the conjecture of the Valdés group, that this novel terpenoid was the main visionary principle of the leaves of Mary Shepherdess. One of the 'basement shamans,' evidently the first human being to ingest pure salvinorin A, then went public, describing *"Salvia divinorum* and salvinorin A: New pharmacologic findings" in the pages of the *Journal of Ethnopharmacology* (Siebert 1994).

In his paper, Siebert briefly described the effects in 6 volunteers of aqueous suspensions of fresh *Salvia divinorum* leaves along with *coca-like* quids of masticated leaves held in the mouth; and of pure salvinorin A in 20 volunteers, administered both by buccal spraying of an ethanolic solution of the compound, and by inhalation through a glass tube of the pure compound vaporized on tinfoil with a butane 'micro torch' (the high melting point of salvinorin A, around 240 C, makes effective vaporization difficult without such an apparatus). The plant material studied was the famous 'Wasson clone.' When subjects were given an aqueous suspension of 10 fresh leaves (about 30 g) homogenized in a blender in 100 ml water, which they then swallowed, followed by rinsing the mouth to minimize contact of the suspension with oral mucosa, "none of the (6) volunteers reported any noticeable effects." When the same suspension was held in the mouth for 10 minutes absent swallowing, then spit out, "all of the volunteers report(ed) very definite psychoactive effects." When doses as high as 10 mg of salvinorin A were swallowed in gelatin capsules "there was no detectable activity." On the other

hand, buccal spraying of 1 ml of ethanol in which 2 mg salvinorin A was dissolved "proved to be active" but weakly so: "this method was inefficient and results were inconsistent." Extraordinarily high activity was found for inhaling the vapors of salvinorin A: "typically threshold effects are noted at about 200 µg (mcg)" and "when 200-500 µg (mcg) of salvinorin A is vaporized and inhaled the subjective effects produced are identical to those typically produced by the fresh herb. Doses up to 2.6 mg were tested in this manner." (Siebert 1994) The pharmacodynamics varied greatly by method of ingestion. The quid method of chewing the leaves provoked first effects in 5-10 minutes which quickly built up to a peak, maintaining a plateau for 1 hour, with effects subsiding over another hour. Inhalation of the vaporized, pure compound led to full effects within 30 seconds, lasting 5-10 minutes, then subsiding over 20-30 minutes. Like Valdés, Siebert stressed the potent and vivid visionary effects:

> "Frequently people report having seen visions of people, objects, and places. With doses above 1 mg, out of body experiences are frequent ... The volunteers who were experienced with other hallucinogens all agreed that despite some similarities, the content of the visions and the overall character of the experience is quite unique."

Siebert also submitted a sample of salvinorin A for screening on neural and other receptors, using a procedure called the Nova-Screen™. In tests of competitive inhibition of binding of reference target compounds, at concentrations of 10^{-5}M, there was no significant inhibition in receptor affinity of the target compound for 40 receptors, including 15 neurotransmitter receptors. This suggests what one would expect, given the novel structure of the compound and its unique effects—that it binds to some other, possibly new, receptor. Siebert concluded that salvinorin A, when swallowed, "is deactivated before entering the blood stream," and that absorption must take place in the buccal mucosa for oral activity. He suggested that injection might result in a threshold of activity yet lower than the 200 mcg following inhalation of the vapors (Siebert 1994). Even as such, salvinorin A is at least an order of magnitude more potent than any other known natural entheogen, such as psilocybine from María Sabina's mushrooms (oral threshold of psilocybine in human beings is about 2 mg (Fisher 1963)), and is within the range of activity of the semi-synthetic ergoline compound lsd. To think María Sabina had characterized *ska Pastora* as lacking strength compared to her beloved mushroomic *children* (Estrada 1977), while the crude mouse assay employed by the Valdés group had suggested that salvinorin A was of the same order of activity as mescaline, a compound which is in fact more than 1000 times less active (Ott 1993)!

On the other hand, it appears Siebert went beyond his evidence in alleging absorption in buccal mucosa was a requisite for activity of the drug. It seems logical that *crystalline* salvinorin A in capsules might not dissolve in gastric juices, thus explaining the inactivity of capsules with high amounts of the pure compound. Although swallowing the homogenate of 10 leaves mechanically blended in water evinced no detectable activity, this observation does not warrant concluding lack of gastric absorption of the drug as prepared in infusions by the Mazatecs. In the first place, this dose is far too low. Although Wasson and Anita Hofmann each felt *mild* effects from a suspension of merely 6 leaves, Albert Hofmann felt next to nothing with the 10-leaf dose utilized by Siebert. We must recall that Valdés had described the dose range as 20-80 pairs of leaves; Gomez Pompa as 8-12 pairs; Weitlaner and Díaz as 25-50 pairs, while Karl Herbert Mayer

mentioned 13 pairs (Mayer 1977)—even Valdés' 'beginner's dose' of 20 pairs is fully *four times* the amount tested by Siebert, whose negative results can thus in no way be construed as proving lack of gastrointestinal absorption. Also, it is not certain that mechanical blending of the leaves in water accurately reproduced the curious method of 'rubbing' the leaves in water employed by the Mazatecs. Indeed, Valdés later characterized this as "a pharmaceutically elegant way of preparing a microsuspension or emulsion of salvinorin A," noting the traditional method was "much more effective than the crude emulsion that was made to dose the mice" in his laboratory experiments (prepared by dissolving salvinorin A in corn oil and surfactant Tween-80, then shaking in water; which emulsion would readily 'break'—this suspension was then injected intraperitoneally into the mice) (Valdés 1994b). Valdés took issue with Siebert's conclusions regarding gastrointestinal absorption of salvinorin A: "from these animal studies one can conclude that the emulsion of the compound allows regular peritoneal absorption," speculating that "although not as potent as inhalation of the vaporized compound, the effects might last longer" noting that in Mexico he had experienced much longer-lasting effects than those reported by Siebert. Indeed, all of the ethnographic reports describe making an infusion of the 'rubbed' fresh leaves in water, which is simply swallowed, with no emphasis on retaining the material in the mouth as long as possible, and only Wasson described the alternate method of simply chewing the leaves, although American anthropologist Bret Blosser independently documented this ingestion method among contemporary Mazatecs (Blosser 1991-1993), as did Mayer (1977) (Blosser added the detail that the stack of pairs of leaves was rolled into a taco or cigar to facilitate chewing the leaves). On the other hand, it is a noteworthy fact that, as Siebert's experiments with a marginal dose of 10 leaves blended in water did show conclusively, buccal absorption is the more effective method of ingestion. To be sure, in the course of chewing 20-80 pairs of fresh leaves, the leaf matter would needs be in contact with buccal mucosa for an extended period, allowing buccal absorption ... but why did the Mazatec Indians fail to discover the obvious advantages of the quid method? This question is especially pointed in that, as Pendell noted: "by the eighth swallow of the leaves the gag reflex becomes overwhelming" (Pendell 1995). Valdés offered an explanation at least for the failure of Mazatec shamans to note the activity of *dried* leaves, suggesting that:

> "Drying drastically alters the chemical composition of the leaves, and the microsuspension/emulsion of salvinorin A will not be formed. Since salvinorin A is insoluble in water, the dry leaves will not serve to prepare an effective infusion." (Valdés 1994b)

On the other hand, Dale Pendell described preparing dried leaves for eating:

> "Salvinorin is practically insoluble in water. The best way to 'ingest' dried leaves is to soften them with some hot water, then keep these leaves in the cheeks just as with fresh material." (Pendell 1995)

The quid method and the preparation of smokeable precipitates from extracts of the leaves were rapidly communicated to the entheogenic underground by Internet b.b.s. and publications like *The Entheogen Review.* In winter 1993 a reader commented that blended juice of 150 fresh-frozen leaves was inactive in three individuals (that is, 50 leaves each), with editor Dekorne noting "without any first hand experience to go on, I can't comment"

(Anon. 1993a). Six months later, another reader described having heard about the quid method (yet another, fresh from a Botanical Preservation Corps seminar in Hawai'i, where Dale Pendell spoke on *Salvia divinorum* and where Dale, Dennis McKenna, myself and others were experimenting with smoking pure salvinorin A that I'd isolated just prior to the event, detailed this quid method) (Anon. 1993b). A year later, Valdés himself had written to the newsletter (Valdés 1994a), warning readers of the reputed "*extreme* potency" of salvinorin A, while one intrepid reader reported making *ayahuasca* analogues (Ott 1994) with *Salvia divinorum* (chewing 6 g of *Peganum harmala* L. seeds with 45 half-dried leaves, reporting an eight-hour experience, describing it as "by far the worst tasting entheogen, though it's my favorite"); and yet another described "spooky ... complete dislocation" from smoking "two or three consecutive bong hits" of dried leaves, giving an effect lasting no more than 20 minutes (Anon. 1994a). Editor Dekorne was prompted to warn his readers:

> *"A Word to the Wise:* Information soon to be made public (a veiled reference, apparently, to Siebert's paper) will almost certainly result in the dea (U.S. Drug Enforcement Administration) putting *Salvia divinorum* on the schedule-1 (most restricted drugs) list, so get it while you can. There's far more to this plant than meets the eye." (Dekorne 1993).

even though his book *Psychedelic* (sic) *Shamanism,* published the following year, characterized the plant as a 'minor psychedelic' and contained a distillate of incorrect speculations about the purported inactivity of dried or frozen leaves, the "extreme instability" of the active agent, etc. (Dekorne 1994). Issue No.6 of the hybrid drug/shamanism magazine *Psychedelic Illuminations* featured a sidebar on "Mazatec Magic," in which the quid method of chewing *Salvia divinorum* leaves was described, as was smoking of the dried leaves, "for milder effects" (Anon 1994b).

This sudden burst of pharmacological activity by the 'basement shamans' evidently alarmed Valdés who, apart from his abovementioned warning to readers of *The Entheogen Review,* published a paper in *Journal of Psychoactive Drugs,* noting:

> "Until recently, *S. divinorum* was considered to be a plant with low abuse potential (sic) ... it is apparent that both *S. divinorum* and salvinorin A are prime candidates to become drugs of widespread use once knowledge of their effects spreads. A small investment in fertilizer and solvents, with only a minimal need for mastery of laboratory technique, would make cultivation of *S. divinorum* and isolation of salvinorin A potentially much more attractive than trying to synthesize lsd or phencyclidine derivatives." (Valdés 1994b)

First we had Dekorne, presumably not in favor of prohibiting entheogens, suggesting prohibition of *Salvia divinorum* to the authorities; then Valdés, presumably opposed to non-traditional use of entheogens, suggesting the idea of cottage-industry, commercial cultivation of *Salvia divinorum* and isolation of salvinorin A for sale on the black market! Valdés even offered useful practical advice, if not detailed instructions, to the would-be black-market producer of *Salvia divinorum* and salvinorin A:

> "Having 80 to 100 12-inch pots (5 cuttings/pot) arranged quincuncially in an area of 4x4 m (12x12 ft), indoors (on benches under normal cool-white fluorescent lighting) or outdoors, can yield well over one kilogram per month of dried leaves once the plants are established (about two to three months) ... An underground chemist, however, would not need to be so meticulous. There is no need for using a

> Soxhlet apparatus, and experimenting could lead to the use of commonly available solvents for the extraction. Yields of even a gram per kilogram of dried leaves would produce some 2,000 human doses." (Valdés 1994b)

Valdés' paper was rather a review of the state of knowledge on *Salvia divinorum* than a report of any new results from his own research. Unfortunately, this was marred by several mistakes. Besides the abovementioned misattribution of the quid method to Mazatec informants of Bret Blosser, who learned of this from Americans in Los Angeles, not from his informants in the Sierra Mazateca (Blosser 1991-1993), Valdés erroneously summarized Siebert's findings with vaporized salvinorin A. He stated that:

> "A dose of *200-500* mcg produces visions that last from 30 minutes to an hour or two, while doses over 2 mg are effective for much longer." (Valdés 1994b)

On the contrary, Siebert stated the full effects were experienced in 30 *seconds,* the duration of the strongest effects was only 5-10 minutes, with the effects subsiding over the following 20-30 minutes (with "somewhat increased" duration at doses above 1 mg). Valdés also weighed in with authoritative opinions on alleged "inaccuracies about *S. divinorum* that are fixed in the literature," to wit: the question of the identity of *Salvia divinorum* with the Nahua entheogen *pipiltzintzintli,* and the purported status of *Salvia divinorum* as a cultigen, rather than as a wild plant. Valdés dismissed both out-of-hand, as "inaccuracies," offering, however, only opinions and no evidence whatever to the contrary. Let us examine both these theories in turn.

With regard to the possible pre-Columbian Nahuatl name for *Salvia divinorum* , Valdés stated authoritatively that:

> "It has been demonstrated that either marijuana or one of various species of morning glories are better candidates (than *S. divinorum)* for being the unknown Aztec plant *pipiltzintzintli)."*

citing his own 1987 paper and Díaz's 1979 review article. Valdés and Díaz, far from demonstrating anything of the kind, merely cited Aguirre Beltran's argument, based on his interpretations of the archives of the Inquisition, that *pipiltzintzintli* was another name for *ololiuhqui* ('round things,' the ergoline-alkaloid-containing seeds of the 'snake plant,' coaxihuitl, *Turbina corymbosa)* (Aguirre Beltran 1963). Since the archives made reference to the use of parts of *pipiltzintzintli* other than simply the leaves, Valdés hastened to note that leaves and stems, as well as seeds, of *T. corymbosa* likewise contained alkaloids. Yet only ground *ololiuhqui* seeds are reportedly used to prepare visionary infusions in Mexican shamanism, and we can readily discard *ololiuhqui* as a possible identity for *pipiltzintzintli* by quoting our primary source on the identity of the mysterious entheogen, 17th century friar Agustin de Vetancurt, who described *the leaves* of *pipiltzintzintli* thus:

> "Tómanla bebida para no sentir cansancio, y aplicadas por modo de emplasto cura las partes desconcertadas, en el agua ordinaria ... y aunque los Naturales las estiman, los Españoles las aborrecen por supersticiosas, porque aquéllos las suellen tomar para adivinar, y saber lo oculto en sueños, mézclase con zacazili, y ololiuhqui para las fracturas. (They take it as a drink so as not to feel weariness, and applied as a poultice they cure injured parts, in ordinary water ... and although the Natural Ones (Indians) esteem them, the Spaniards abhor them as superstitious because those people

> are wont to take them for divination, and to learn hidden things in dreams, mixing them with *zacazili* and *ololiuhqui* for fractures."

So *pipiltzintzintli* was mixed with *ololiuhqui*—it is thus obvious that we are dealing with two different drugs *(zacazili* may correspond to *sacasil,* a species of *Anredera,* or to *sacasile, Boussingaultia* sp. (Díaz 1976))! Since *pipiltzintzintli* had both male and female varieties, and was also used dried, both Díaz and Valdés suggested marijuana, *Cannabis* spp. as a "likely candidate." This suggestion is frivolous—rather like speculating that *soma* or Homer's *nepenthes* was *peyotl!* While there exists taxonomic debate over the question of speciation in *Cannabis* (Ott 1993), there is no question of the Eurasian origin of *Cannabis,* botanists universally regard it to be a post-contact introduction to the New World, and noted experts Richard Evans Schultes and Albert Hofmann diplomatically dismissed Díaz's and Valdés' proposal as being "more than highly unlikely" (Schultes & Hofmann 1980).

Pendell cited the lack of sexes in *Salvia divinorum* as militating against its identity with *pipiltzintzintli,* but as he himself allowed, "it is also possible that the reference to gender is metaphorical" (Pendell 1995), as is certainly the case with male/female pairing of entheogenic mushrooms used shamanically in various parts of Mexico (Ott 1993; Rubel & Gettelfinger-Krejci 1976; Wasson & Wasson 1957); likewise with male and female elements of plant combinations in Amazonian *ayahuasca* potions (Ott 1994). Furthermore, Wasson's pioneering paper noted exactly this with respect to the leaves of the Shepherdess, said by his Mazatec informants to be 'the female' in a 'family' including 'the male,' *Coleus pumilus,* and a 'child,' *C. blumei* (Wasson 1962). The lack of botanical sexes in *Salvia divinorum* constitutes specious grounds to reject the identity of this drug with *pipiltzintzintli,* given the common use of sex-pairing as a metaphor for entheogenic plant ingestion or dosing; and the fact that Vetancurt described both the drinking of a potion of *pipiltzintzintli* for divination, and application of the leaves used to make the potion as a poultice—precisely what Valdés himself reported for Mazatec use of *Salvia divinorum* (while Weitlaner reported similar cutaneous application of the potion itself)—argues eloquently for Wasson's proposal that *pipiltzintzintli* was *Salvia divinorum.* Can Valdés point to any other Mesoamerican entheogen whose leaves are used to prepare a divinatory potion, and also applied cutaneously as a remedy? In the case of *ololiuhqui,* Sahagun and Hernandez described divinatory use of potions, and the therapeutic, cutaneous application of same, but prepared from the seeds, and not from the leaves of the plant (indeed, *ololiuhqui* is the Nahuatl name of the seeds only, the plant is called *coaxihuitl* or *coatlxoxouhqui*—'snake plant' or 'green snake' (Ott 1993)).

Garza mentioned the use in Tepoztlán, Morelos of a plant called *piltzintzintli,* a vine with pods full of red-and-black seeds, which seeds were taken daily, one at a time, up to a total dosage of 12, to treat 'airs' (Garza 1990). While she was unable to identify this plant botanically, it surely corresponds to the well-known *Rhynchosia spp.*—Díaz noted that *Rhynchosia* species, with brilliant red-and-black seeds borne in pods, are known as *pipiltzintli* in northern Mexico (Díaz 1979). The Wassons described divinatory use of six pairs of seeds of *Rhynchosia pyramidalis* (Lam.) Urban, combined with 6 pairs of the psilocybian mushroom *Psilocybe aztecorum* Heim, known as *apipiltzin* ('little children of the waters') by a Nahua *curandera* in San Pedro Nexapa, high on Popocatepetl (Wasson & Wasson 1957). This sounds like a promising lead, but the *Rhynchosia* seeds were known descriptively in San Pedro Nexapa as 'bird's eyes,' not as *pipiltzintzintli,* and of

this mysterious Aztec entheogen, it is the seeds which were not mentioned as being used, as Valdés admitted (Valdés et al. 1987a). Of course, this further militates against the misidentification of *ololiuhqui* as the lost Aztec drug, but is consistent with the relatively seedless (as we will see below) *Salvia divinorum* being *pipiltzintzintli.*

We have thus seen that, far from 'demonstrating' better candidates than *Salvia divinorum* for *pipiltzintzintli,* Valdés has offered one that is impossible, not having been present in pre-Columbian Mexico, and another which our primary source clearly identified as a plant distinct from *pipiltzintzintli,* and that was in fact mixed with it to treat fractures! After this inauspicious start, Valdés fared no better in his 'demonstration' of the second alleged 'inaccuracy' in the literature, the status of *Salvia divinorum* as cultigen. Wasson had stated that (Wasson 1962):

> "We were on the watch for *Salvia divinorum* as we criss-crossed the Sierra Mazateca on horseback in September and October of 1962, but never once did we see it. The Indians choose some remote ravine for the planting of it and they are loath to reveal the spots ... *Salvia divinorum* seems to be a cultigen; whether it occurs in a wild state (except for plants that have been abandoned or have escaped) we do not know."

I noted in my 1993 book *Pharmacotheon,* also singled out by Valdés, that (Ott 1993):

> "The Mazatec Indians believe the plant is foreign to their region of the Sierra Madre Oriental and we do not know whence it came, as no wild populations have been discovered ..."

In arguing against this, Valdés only offered the unverified statement of his informant Don Alejandro, that "the plant grows wild in the fairly inaccessible highlands of the Sierra Mazateca," and described seeing large stands along a creek in a small ravine and in a coffee plantation (Díaz (1975) gave the altitude range of *Salvia divinorum* as 750-1500 m; but Wasson (1962) described it as growing in Huautla de Jiménez at 1800 m, and Don Alejandro's claim might be construed as suggesting it grows near the 2100 m summit of Cerro Rabon) (Valdés 1994b; Valdés et a1. 1987a). What Valdés actually observed is not inconsistent with either Wasson's statements or my own (he admitted the stands he saw were "apparently originally started by humans"), and absent documentation of the purportedly wild stands described by Don Alejandro, he has given us no evidence that the plant exists in truly wild conditions. He further cited Siebert's recent collection of viable seed from cultivated *Salvia divinorum* in Hawai'i as evidence of its wild nature (Siebert 1993-1994), but a recent botanical and horticultural study not cited by Valdés supports Wasson's contention that the plant is a cultigen (Reisfield 1993). Following up Valdés successful production of seed from cross-pollination of two strains of *Salvia divinorum,* Reisfield was also able to obtain viable seed from self-pollinated strains of the plant, but both manual cross- or self-pollination had extremely low success rates (only a few percent). Reisfield suggested the plant was a hybrid, possibly of largely incompatible parents which remain unknown. He could cite no prospective parents, and Epling and Játiva merely compared *Salvia divinorum* to the central Mexican *S. cyanea* Lamb. ex Benth., a species recently collected by Siebert and analyzed for salvinorin A, with negative results (Siebert 1993-1994). Since 1991, I have been growing 3 different strains of *Salvia divinorum* (91-11, the 'Wasson clone' from San José Tenango at 1200 m altitude, and 91-41 and 91-42, two so-called 'palatable clones' collected by Bret Blosser

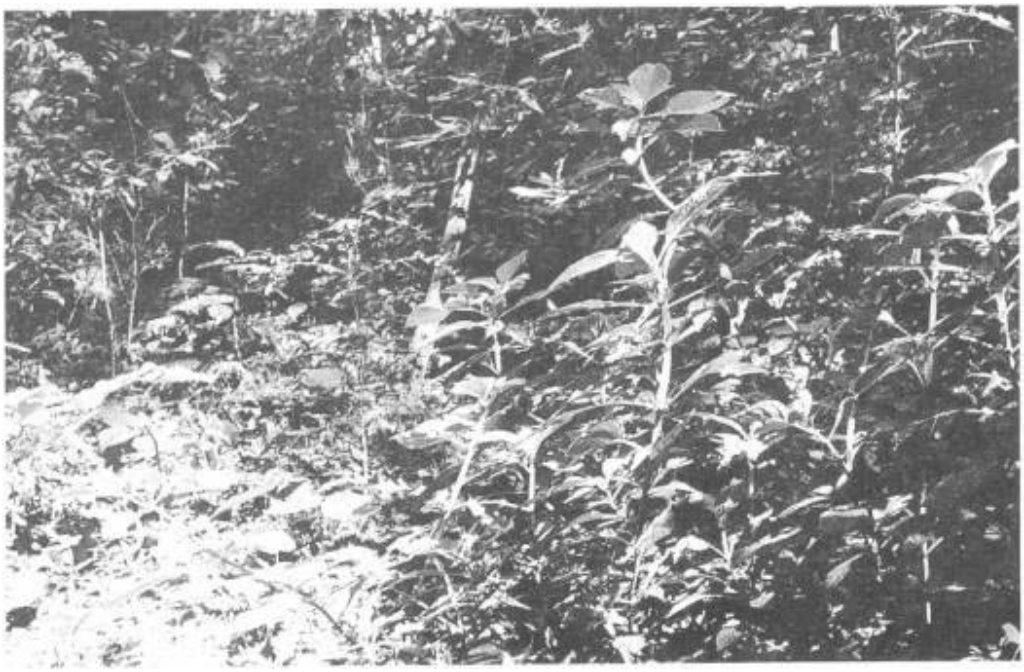
in Llano de Arnica, Municipio de Tenango, Oaxaca) side-by-side in a natural setting near Xalapa, Veracruz (at 1350 m altitude, about 150 km north of Huautla de Jiménez). All have prospered, flowered abundantly and repeatedly, but no seed has set, despite repeated attempts at manual self- and cross-pollination. Unless Valdés can document Don Alejandro's contention that *Salvia divinorum* in fact grows wild in inaccessible areas of the Sierra Mazateca, the best conclusion we can draw from the available evidence is that, as Wasson stated from the outset, the plant is a cultigen.

I would also like to point out another inaccurate statement Valdés made with regard to *Salvia divinorum*—in his paper describing his isolation of salvinorin A, he claimed it was "the first clearly documented psychotropic terpenoid" (Valdés et a1. 1984). In fact, the psychotropic terpenoid thujone (Merck Index 11: 9326; synonyms: absinthol, salvanol, tanacetone), active principle of wormwood, *Artemisia absinthium* L. and the famous *absinthe* liqueurs distilled from it, has been known for nearly a century; and the psychotropic terpenoid cannabinols (Merck Index 11: 9142) from *Cannabis spp.* for more than three decades. Thujone even occurs in high concentrations in some strains of culinary sage, *Salvia officinalis* L. (Tucker et al. 1980), and *smelling* that plant can have psychoactive sequelae, as thujone is volatile (Duke 1987). Steam distillation of fresh leaves of *Salvia divinorum* showed they contained no thujone (Ott 1993). One of the well-known pre-Columbian entheogens is *itzauhyatl, Artemisia mexicana* Willdenow, a probable thujone-containing species, and psychoactive *Artemisia* species were widely used by Native Americans (Ott 1993). The Oraon tribals of West Bengal, India, were recently reported to smoke leaves of the thujone-containing (Uniyal et al. 1985) *Artemisia nilagirica* (Clarke) Pamp. as an entheogen (Pal & Jain 1989). In Amazonia, the mint *Ocimum micranthum* Willdenow is considered to be entheogenic (Duke & Vasquez 1994), and is known to be added to *ayahuasca* potions (Ott 1994). As for the *Coleus* species said to belong to the same 'family' as *Salvia divinorum, Coleus blumei* is known to contain terpenoids (García et al. 1973), flavonoids and coumarins (Lamprecht et al. 1975) of unknown psychopharmacology. Terpenoids known as coleones are found in other species of the genus (Arihara et al. 1975), and *Coleus blumei* was shown not to contain the hypotensive terpenoid colforsin or forskolin (Shah et al. 1980), found in the Ayurvedic medicine gurmal or *Coleus barbatus* (Andrews) Bentham (Valdés et al. 1987b). Along with *Coleus blumei* and C. *pumilus,* the well-known Ayurvedic medicine *pashnabhedi, Coleus amboinicus* Lourteig (Nadkarni 1976) might be a good candidate for screening for salvinorin A or allied compounds—in the classic text, *Indian Medicinal Plants,* it is stated (Kirtikar et al. 1918):

> *"In spite of its intoxicating properties* the people of Bengal employ it in colic and dyspepsia." (italics mine)

Before summarizing the human pharmacology of *Salvia divinorum* and salvinorin A, I would like to list my reasons for regarding the shamanic use of this drug to be a post-Conquest innovation in the Sierra Mazateca. I had previously mentioned the lack of a truly indigenous name for *Salvia divinorum* among the Mazatecs. It is suspicious that the Mazatecs associate the plant with the Biblical Mary, and with sheep, both post-Conquest introductions to the Sierra Mazateca, and Valdés documented remedial use of infusions of 4-5 pairs of *Salvia divinorum* leaves to treat a disease called *panzón de barrego* (sic), 'big lamb's belly' (Valdés et al. 1983). We also have the precedent of the mushroom

Psilocybe cubensis (Earle) Singer, introduced to Mexico by Europeans along with the cattle in whose dung it grows. Some Mazatec *curanderos* have come to ultilize this mushroom as a shamanic inebriant, others eschew it (and, tellingly, those who do use it hold it to be the 'least esteemed' species). This is exactly what we find with *Salvia divinorum*—we have seen that María Sabina held it in low esteem. Like the leaves of Mary Shepherdess, *P. cubensis* lacks a truly indigenous name, being known prosaically in Mazatec as the 'sacred mushroom of the bull's dung'; or in Spanish as *honguillo de San Isidro Labrador,* the 'mushroom of St. Isidore the Plowman,' patron saint of Madrid! (Wasson & Wasson 1957). The fact that the Mazatecs put *Salvia divinorum* in the same 'family' as two species of *Coleus* known to be post-Conquest introductions to Mexico is further evidence for this hypothesis. What clinches the argument for me, however, is how little the Mazatecs seem to know about using the drug. They believe the leaves to be inactive when dried, but this is not true—the dried leaves preserve their activity indefinitely and salvinorin A is highly stable. Valdés suggested the dried leaves were unsuitable for preparing the aqueous infusion, but Pendell has shown they can be successfully rehydrated for oral ingestion, one way the Mazatecs have been documented using the fresh leaves. Valdés saw in the strange method of preparing an infusion of the fresh leaves: "a pharmaceutically elegant way of preparing a microsuspension or emulsion of salvinorin A," while Wasson dismissed this as "certainly an inefficient method." Siebert's studies showed it to be indeed an inefficient method—a marginal, low dose which provoked no effects in an imitation of the Mazatec technique (and the same dose which was all but inactive for Albert Hofmann, even when prepared under the supervision of María Sabina) was "consistently effective" at evoking "definite psychoactive effects" utilizing the simple quid method, readily discovered by American 'basement shamans,' but not divined by the Mazatecs. Far from being an 'elegant way' of ingesting the leaves of *Salvia divinorum,* this seems rather a crude adaptation of the standard Mazatec (and other Mesoamerican Indian) technique for preparing the psilocybian mushrooms and the entheogenic morning glory seeds, which are traditionally crushed on a *metate* and infused in water (Wasson 1963). It is as 'though the Mazatecs had adapted this standard technique for processing entheogenic plants for ingestion, which is indicated in the case of the mushrooms and seeds, but barely effective in the case of the leaves ... as 'though they had learned comparatively lately of this drug, which was given a name inspired by the religion and economy of their conquerors, and to process which they simply adapted their existing technique for processing entheogens, despite the fact that it hardly works in this novel case. So ineffective is this adapted processing, that the leaves of Mary Shepherdess have the reputation among the Mazatecs of being much less powerful than the psilocybian mushrooms. Even Valdés' informants regarded *Salvia divinorum* to be weaker than the morning glory seeds or the mushrooms (Valdés et al. 1983). Hofmann found 0.2% psilocybine (dry weight) in cultivated *Psilocybe caerulescens* Murrill from a strain collected in July 1956 in Huautla de Jiménez (Heim & Hofmann 1958), while Valdés isolated 0.18 % salvinorin A from dried leaves of *Salvia divinorum*—making the leaves, gram per gram, nearly 10 times as potent as the mushrooms (since salvinorin A is roughly 10 times the potency of psilocybine)! If the Mazatecs have a long familiarity with the leaves, if in reality they have developed a 'pharmaceutically elegant' way of processing them for ingestion, then why do they fail to perceive them as being far and away the most potent entheogen available to them?



Salvia divinorum (Photograph: Jonathan Ott)

Summary of *Salvia divinorum* and Salvinorin A Pharmacology

I. Infusion of Leaves in Water
Dose:
(Weitlaner 1952)
50-100 leaves
(Gómez Pompa 1957)
16-24 leaves
(Wasson 1962)
6-68 leaves
68 leaves (RGW—"dancing colors in elaborate ... designs")
10 leaves (RGW—"we both felt the effects")
10 leaves (Albert H.—"mental sensitivity and intense experience")
(Hofmann 1979)
6 leaves' (Anita H.—"striking, brightly bordered images")
(Roquet 1972)
240 leaves
(Díaz 1975)

50-100 leaves (“far from being hallucinations”)
(Mayer 1977)
26 leaves
(Roquet & Favreau 1981)
32-48 leaves (“dose of 40 to 60 g via oral”)
(Valdés et al. 1983)
40-160 leaves (“or more”—40-120 in Valdés et al. 1987a)
120 leaves (JLD—“the images ... they are weak, no?”)
100 leaves (JLD)
100 leaves (LJV—“the ‘reality’ of what he was seeing amazed him”)
40 leaves (LJV—“there were shapes like pillars of kaleidoscopic smoke”)
8-10 leaves (“tonic” or “panacea” or “placebo” dose)
(Foster 1984)
20 leaves (“the leaves produced hardly noticeable effects”)
(Anon. 1993a)
50 leaves (frozen—“effects... indistinguishable from... imagination”)
(Siebert 1994)
10 leaves (blended—“none... reported any noticeable effects”)

II. Whole Leaf, Swallowed
Dose:
(Pendell 1995)
26 leaves (“it lights up the mouth like a rainbow”)
(Ott 1995b)
26 leaves (“insufficient effects to convince me they weren’t imaginary”)

III. Leaf, Quid Method
Dose:
(Siebert 1994)
10 leaves (blended—“all... reporting very definite psychoactive effects”)
(Anon. 1993b)
18-26 leaves (“ingredient needs to be absorbed through the mouth”)
(Anon. 1994b)
12-16 leaves (“profound visual effects will be noticed with eyes closed”)
(Forte 1994)
26 leaves (“wonderful, sublime, and outrageously funny”)
(Schuldes 1994)
18 leaves (“overwhelming ... nonstop, very powerfull (sic) laughter”)
(Pendell 1995)
6-10 leaves (“or more”—“a deeper and more sustained experience”)

(Ott 1995b)
6 leaves (“definitely psychoactive; far more potent than 26 leaves eaten”)

IV. Dried Leaf Smoked
Dose:
(Ott 1993)
1-2 leaves (“five or six puffs ... mild effect... lasts for one to two hours”)
(Anon. 1994b)
dried leaves (“can be smoked for milder effects”)
(Pendell 1995)
1-2 leaves (“smoking the dried leaves produces immediate effects”)

V. Salvinorin A, Vaporized
Dose:
(Weil 1993)
high dose (“sense of being smothered ... amazingly powerful”)
(Siebert 1994)
200 mcg (“typical threshold effects are noted”)
200-500 mcg (“effects produced are identical to ... the fresh herb”)
1.0-2.6 mg (“doses above 1 mg, out of body experiences are frequent”)
(Pendell 1995)
500-S00 mcg (“about twenty times more active by weight than dmt”)
1.0 mg (“maybe there were some visuals”)
(Ott 1995b)
500 mcg (“threshold level for visionary effects, very rapid and short”)
500-800 mcg (“very enjoyable visionary effects, hyperthermia”)
(Strassman 1995)
1.2 mg (“the fabric of reality does unzip and roll up”)

VI. Salvinorin A, Peroral
Dose:
(Siebert 1994)
10.0 mg (swallowed—“encapsulated ... there was no detectable activity”)
2.0 mg (buccal spray in solution—“active ... inefficient ... inconsistent”)
(Ott 1995b)
100 mcg (1% acetone solution—“threshold for definite physical effects”)

250-500 mcg ("euphoria, auditory and visual effects, colored patterns")
1.0 mg ("pronounced hyperthermia, swirling colored patterns")

Conclusions and Commentary

As can be seen from the above tabular summary, *Salvia divinorum* leaf is active when swallowed in aqueous suspension, when chewed and swallowed, when chewed as a quid, or when dried and smoked; and salvinorin A is active when vaporized and inhaled or when ingested sublingually in solution. The probable *descending* order of potency is as follows:

> sublingual salvinorin A ≥ vaporized salvinorin A ≥ chewed leaf, quid ≥chewed leaf, swallowed ≥ infusions of leaf

I have not attempted to include the smoked leaves in this scheme—even 'though as few as 1-2 leaves may be active, since for most people the effect is much milder than by oral ingestion (albeit of greater quantities). In a test with 20 people, each of whom was given a 'joint' of dried *Salvia divinorum* leaves to smoke (containing 1-2 leaves), roughly half felt nothing at all. Of the half who did feel the effects, all reported quite mild effects, except for 2 individuals, who had potent visionary effects.

It is obvious Siebert was too hasty in concluding *Salvia divinorum* infusions were inactive unless absorbed in the mouth—an infusion of as few as 6 leaves provoked visionary effects. On the other hand, infusions of as many as 120 leaves gave weak visionary effects, and repeated doses in the range of 50-100 leaves provoked effects "far from being hallucinations." This is clearly an inefficient method of ingestion; and we have seen that an infusion of 10 leaves was definitely psychoactive in 6 volunteers by the quid method, while the same strength of an identical preparation was inactive when swallowed by the same volunteers 'several days' later. As few as 6 leaves chewed by the quid method have been reported to provoke psychoactive effects.

As for pure salvinorin A, Siebert reported a threshold of activity at 200 mcg for vaporizing and inhaling the compound, and definite psychoactivity in the 200-500 mcg range; with 1.0-2.6 mg provoking out-of-body experiences. In my own tests, I found a higher threshold of activity, 500 mcg; with 500-800 mcg being the range for definite psychoactivity. Siebert had heated the material on tinfoil and inhaled the vapors through a glass tube; whereas I had placed the compound inside a glass tube for heating and subsequent inhalation of the resulting vapor. While my method, in contrast to Siebert's, virtually guaranteed no loss of 'side-stream' vapor, Siebert's method probably was conducive to more complete vaporization of the compound which, as mentioned above, has a high melting point, around 240 C.

As for oral ingestion of the pure compound, Siebert found 10 mg inactive when swallowed as crystals in a capsule—which makes perfect sense, given the improbability of dissolution of the crystals in gastric juices. Siebert further reported doses as high as 2 mg in 1 ml ethanol solution were indifferently active; whereas I found a threshold of activity for sublingually-applied 1 % solutions of salvinorin A in acetone at 100 mcg,

with 250 mcg-1.0 mg provoking definite visionary psychoactivity. It is easy to explain this discrepancy. Ethanol is not a suitable vehicle, as salvinorin A is not sufficiently soluble therein—Siebert's weak solution (0.2%) probably provoked local irritation and subsequent salivation, further diluting (and perhaps even provoking precipitation of) the salvinorin A, thus preventing efficient absorption. At 1 % strength in acetone, however, 100 mcg of salvinorin A can be delivered in 10 mcl (10 λ) of acetone, which provokes only slight irritation and is readily absorbed before salivation can interfere—the first effects are typically felt within 90 seconds, and reach a maximum within 10-15 minutes. How might we explain my observation of a lower threshold for sublingual, as opposed to vaporized and inhaled, salvinorin A (about half the threshold Siebert found, as little as one-fifth the threshold I found)? When vaporizing and inhaling the pure compound, considerable condensation was evident in the glass tube used to inspire the vapor—once again, the high melting point is the culprit. As the salvinorin A vapor cools on its way through the tube, some condenses inside the tube. Of course, one could control for this by putting the crystals inside the tube, then precisely weighing the tube before and after vaporization, which would give a more accurate picture of the amount of vapor inhaled, and the amount retained in the tube. I suspect that, even if no vapor is lost to the 'side-stream,' only about half makes it through the tube, the remaining half recondensing inside. I speculate that with proper controls, it would be found that salvinorin A is equipotent whether sublingually applied, or vaporized and inhaled—perhaps even more potent in the latter case, although sublingual application of concentrated solutions is a simpler and more healthful method of ingestion. Although I made 1 % test solutions in acetone (10 mg/1.0 ml), concentrations as high as 10% might be possible (100 mg/1.0 ml), further minimizing the amount of solvent involved (in the latter case, 1.0 mg of salvinorin A could be delivered in 10 mcl of solution). Salvinorin A is sufficiently soluble in the aprotic solvent dimethyl-sulfoxide or DMSO, to prepare 1 % solutions, and this solvent was also found to be an effective means of sublingual delivery of salvinorin A.

Of extreme interest in these studies is the finding that salvinorin A is an order of magnitude more potent orally than any other known natural entheogen. Psilocybine has been said to be active in doses as low as 2.0 mg (Eisher 1963), while a tenth that dose of salvinorin A was found to be active in both the present study and that of Siebert. Indeed, only the artificial ergoline alkaloid LSD exceeds salvinorin A in entheogenic potency—doses of only 25 mcg of LSD free-base provoke a definite and longlasting stimulation, and 200 mcg of the free-base provokes potent entheogenic effects. I would estimate salvinorin A to be about one-fifth to one-tenth the potency of LSD free-base. In light of this fact, it is most interesting to note that even Mazatec shamans who seem to specialize in the use of *Salvia divinorum,* and who are also familiar with the effects of the psilocybian mushrooms and of the ergoline-alkaloid-containing morning glory seeds, regard the leaves of the Shepherdess to be the least potent of the three! This fact, combined with the lack of a truly indigenous name for the leaves in Mazatec, and Mazatec use of crude and inefficient methods for preparing the leaves for ingestion (not to mention their association of this plant with a 'family' including two Asiatic *Coleus* species clearly introduced to Mexico after the conquest), leads me to conclude that the shamanic use of the leaves in the Sierra Mazateca is a recent, post-conquest innovation. This, of course, begs the question—whence derived this practice?

I doubt *Salvia divinorum* was (inadvertently) introduced to Mesoamerica by Europeans, although it is ineluctably associated by the Mazatecs with sheep, which of course were. It seems more likely that the plant was used since pre-Columbian times by another group of Mesoamerican Indians. We have seen that Emboden suggested the ancient Maya knew of *Salvia divinorum,* and Wasson proposed that the Nahua peoples of central Mexico were familiar with the plant, and used it for its entheogenic properties, under the name *pipiltzintzintli.* Although of course we cannot prove Wasson's assertion beyond any doubt, we have seen that *Salvia divinorum* fits the available, albeit scanty, evidence, and that none of this evidence would preclude the identity of *Salvia divinorum* and *pipiltzintzintli.* Valdés weakly argued against Wasson's proposed identification, but could only offer *ololiuhqui* (seeds of the 'snake plant,' *coaxihuitl)* or marijuana *(Cannabis spp.)* as alternatives! The latter can immediately be eliminated from consideration, given the established fact that it is an Asiatic plant, and was clearly introduced to Mesoamerica in colonial times. As for *ololiuhqui,* this is the name exclusively of the seeds of the 'snake plant,' which seeds alone are used to prepare entheogenic infusions in Mesoamerica, and the seeds of *pipiltzintzintli* are the one part of the plant *not* mentioned as having been used for entheogenic effects in the annals of the Inquisition and the accounts of Agustin de Vetancurt. Since Friar de Vetancurt informs us that *pilpiltzintzintli* was sometimes taken together with *ololiuhqui,* it is obvious we are dealing with two distinct plants, and *pipiltzintzintli* cannot be *ololiuhqui.* The telling piece of evidence, that *pipiltzintzintli* leaves were used to make visionary infusions and also applied cutaneously as a poultice—precisely what has been observed in the contemporary Sierra Mazateca for *Salvia divinorum*—is an eloquent argument in favor of Wasson's proposed identification. *Salvia divinorum* is the only Mexican entheogenic plant which fits the criteria for *pipiltzintzintli,* and unless Valdés or anyone else can come up with a candidate which better meets these criteria, it remains our best guess for the identity of the lost Aztec entheogen.

I cannot conclude this review without lamenting the failure of Valdés' group to use the Heffter Technique to resolve the psychopharmacology of *Salvia divinorum.* It must be counted as a stroke of luck that their crude mouse bioassay led to the isolation of the visionary principle of the leaves of the Shepherdess. All previous attempts at using animal bioassays for this sort of work failed. In the case of *peyotl,* Lewin was unable to isolate the visionary constituent, despite a lead of several years over his competitor Arthur Heffter, who quickly determined that mescaline was the main visionary principle, on the basis of self-experiments. In the case of the psilocybian mushrooms, the group of James Moore, working secretly for the U.S. CIA (Central Intelligence Agency), despite a lead of two years and access to ton quantites of cultivated mushrooms, again failed with animal bioassays, to be scooped by Albert Hofmann who, with only 100 grams of dried mushrooms and using himself and colleagues as guinea pigs, quickly isolated psilocybine and psilocine. Similarly, with the *ololiuhqui* seeds, chemists working for the CIA again failed to isolate the active alkaloids using animal assays, although again they enjoyed a lead of many years over Albert Hofmann who, guided by psychonautic bioassays, later showed the presence of psychoactive ergoline alkaloids in the seeds (Ott 1993). It is surprising that the mouse bioassay used by Valdés et al. gave useful results, but not surprising that it led to a failure to perceive the extreme potency of salvinorin A—estimating it was equipotent with mescaline, which is in fact at least 1000 times *less*

potent! Due to the inexplicable failure of the Valdés group to complete their research with human testing of salvinorin A, a decade passed in limbo, before non-professional 'basement shamans' completed the missing experiments. We are still left in the dark as to the psychoactivity of salvinorin B or the unidentified "at least two more terpenoids" also isolated from the leaves more than a decade ago! While I am not so extreme as to argue that all use of animals as experimental subjects is immoral and unjustifiable, there is no question that the use of mice by the Valdés group was immoral. Although the mice were evidently not killed by the high doses of *Salvia divinorum* and extracts given them, up to 1.0 g/kg salvinorin A (equivalent to 70 grams or 700,000 times the threshold dose in a 70 kg human being!), generally speaking any animals used in pharmacological tests are later 'sacrificed' as being no longer 'naive,' and in any case these animals were clearly bred in capitivity and then used and disposed of at the whim of their human captors. Given the fact that the most effective, and the *only* ultimately valid bioassay to guide isolation of entheogenic compounds is the human, psychonautic bioassay, the Heffter Technique, there is no technical or moral reason to justify abusing captive animals in this manner—Valdés cavalierly noted "all animals survived and appeared unharmed, but they were not autopsied" (Valdés 1994b)! I must also stress that, as both the Shulgins and I have argued, the only ethical way to conduct this sort of research is for the principal investigator first to test any preparation for activity or toxicity, and subsequently to use only free, fully-informed volunteers for further testing (Ott 1993; Shulgin & Shulgin 1991). Had this ethical procedure been followed, not only would animal suffering have been averted, but the technical problem of the visionary principle of *Salvia divinorum* would have been solved an entire decade sooner than it in fact was! Perhaps Valdés can redeem himself now, by testing salvinorin B and the other pair of terpenoids he isolated from *Salvia divinorum* in self-experiments, later publishing the results …

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