

Published in final edited form as:

Phytochemistry. 2013 July ; 91: 229–235. doi:10.1016/j.phytochem.2012.02.021.

Labdane Diterpenoids from *Leonotis leonurus*

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Abstract

Three known (leoleorins A-C) and eight new (leoleorins D-J and 16-*epi*-leoleorin F) labdane diterpenoids, were isolated from leaves of *Leonotis leonurus*. The absolute configurations of leoleorins A and D were established by X-ray crystallographic analyses. In competitive binding assay all isolated compounds showed inhibition in excess of 50% at various CNS receptors. Leoleorin C showed moderate binding affinity ($K_i = 2.9 \mu M$) for the Sigma 1 receptor.

Keywords

Leonotis leonurus; Lamiaceae; Psychoactive plant; Labdane diterpenoids; Leoleorins A-J; X-ray diffraction; G-protein-coupled receptors

1. Introduction

Leonotis leonurus (L.) R. Br. (Lamiaceae), also known as Lion's Tail or Wild Dagga, is commonly used in Southern Africa to treat fevers, headaches, dysentery, flu, chest infections, epilepsy, constipation, delayed menstruation, intestinal worms, spider bites, scorpion stings, hypertension, and snake bites (Iwarsson, 1985; Stafford et al., 2008). It is also known for its mild psychoactive properties. The dried leaves and flowers have a mild

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Appendix A. Supplementary data

Supplementary data associated with this article (The ¹H, ¹³C NMR spectra of compounds 1–8, and crystallographic data for 1 and 9 and primary binding results in biological test) can be found, in the online version,

calming effect when smoked, which is similar to the effect of the THC cannabinoid found in Cannabis. It has also been reported to cause mild euphoria, visual changes, dizziness, nausea, sweating, sedation, and light headedness. The picked and dried leaves are also commonly brewed as a minty tea. Research showed that it could be used to benefit people with anxiety and depression due to its calming effects. *L. leonurus* has been proposed to modulate dopaminergic system (Stafford et al., 2008). Water and lipid extracts of the leaves showed anticonvulsant (Bienvenu et al., 2002), antinociceptive (Ojewole, 2005), anti-inflammatory (Ojewole, 2005; Stafford et al., 2005), antidiabetic (Ojewole, 2005; Oyedemi et al., 2011), antibacterial (Stafford et al., 2005; Scott et al., 2004; Jimoh et al., 2010), anti-oxidant (Jimoh et al., 2010; Frum and Viljoen, 2006), and anthelmintic (Maphosa et al., 2010) activities.

Previous phytochemical analysis of extracts of *L. leonurus* indicated the presence of tannins, alkaloids, as well as steroidal and triterpenoid saponins (Bienvenu et al., 2002). Organic extracts of the flowering parts of the plant provided flavonoids and acyclic diterpene esters (El-Ansari et al., 2009; Agnihotri et al., 2009), while the leaves contained mostly labdane diterpenoids (Cragg and Little, 1962; Rivett, 1964; Kaplan and Rivett, 1968; Laonigro et al., 1979; Kruger and Rivett, 1988; McKenzie et al., 2006; Obikeze et al., 2008; Naidoo et al., 2011). Herein, the isolation and structure elucidation of eight new (1-8) and three known (9-11) labdane diterpenoids (Fig. 1) from this species are reported. In collaboration with the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP), in vitro receptor screening of all pure isolates resulted in identification of the active constituents of *L. leonurus*.

2. Results and discussion

2.1 Structural elucidation of compounds

Leoleorin D (**1**), was obtained as colorless crystals, $[\alpha]_D^{23} -7.5$, and was established to have a molecular formula of $C_{20}H_{36}O_4$ by HR-ESIMS (m/z 363.2499 $[M + Na]^+$). In the 1H NMR spectrum, singlets representing three tertiary methyl groups at δ_H 1.01, 1.24, and 1.27, and a doublet of one secondary methyl group at δ_H 0.93 ($J = 6.8$ Hz) were evident. The ^{13}C NMR and DEPT spectra exhibited 20 carbon resonances ($4 \times C$, $3 \times CH$, $9 \times CH_2$, and $4 \times CH_3$). There were signals of two oxygenated quaternary carbons at δ_C 94.1 and 85.3, characteristic features of the oxygenated carbon of a spirotetrahydrofuran moiety, two oxymethylene groups [δ_C 66.8; δ_H 3.50 (H-16a, d, $J = 10.8$ Hz) and δ_H 3.62 (H-16b, d, $J = 10.8$ Hz); δ_C 59.6; δ_H 3.85 (H₂-15, m)], and one oxymethine group [δ_C 68.0; δ_H 4.36 (br s)]. The molecular formula requiring three degrees of unsaturation, in conjunction with the absence of UV absorption and sp^2 or sp carbon resonances, suggested a highly oxygenated tricyclic labdane diterpenoid skeleton for **1**, similar to the structure of lagochilin (Chizhov et al., 1979). In the HMBC spectrum, correlations of H-6 with C-4, C-5, C-7, C-8, C-10, Me-17 with C-7, C-8, C-9, Me-20 with C-1, C-5, C-9, C-10, as well as Me-18 and Me-19 with C-3, C-4, and C-5, were used to assign the hydroxy group to C-6. The known α -orientation of H-5 in labdane diterpenoids (Wu et al., 2011), and the coupling profile (br s) of H-5 and H-6 indicated the *cis*-arrangement of these two protons in an α -axial, α -equatorial orientation, with the 6-OH β -axially oriented. Thus, the structure of **1** was proposed as 9,13-epoxylabdane-6 β ,15,16-triol. X-ray crystallographic data of **1** (Fig. 2) further defined its absolute configuration and structure as (5*S*,6*R*,8*R*,9*R*,10*S*,13*S*)-9,13-epoxylabdane-6,15,16-triol.

Leoleorin E (**2**), was isolated as a colorless oil, $[\alpha]_D^{23} -3.3$, and its molecular formula was determined to be $C_{20}H_{34}O_4$ by HR-ESIMS (m/z 361.2351 $[M + Na]^+$). The 1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated the presence of a C-15 hemiacetal

carbon (δ_{C} 98.9, CH; δ_{H} 5.41, d, $J = 4.8$ Hz) rather than a C-16 hemiacetal, due to the presence of the H₂-16 resonances at δ_{H} 3.55 (d, $J = 8.8$ Hz) and 4.27 (d, $J = 8.8$ Hz). The HMBC correlations showed similar relationships as in **1**, except for the presence of correlations between H₂-16 and C-15, and H₂-15 and C-16. The relative configuration of **2** was assigned on the basis of NOESY correlations (Fig. 3). Based on the assumed 5*S* configuration for labdane diterpenoids and the NOEs between Me-17 and H₂-14 and between H-16a (δ_{H} 4.27) and H-1 α (δ_{H} 1.89), which are consistent with a 13*S* configuration of the spirocyclic D-ring. The β -orientation of H-15 was concluded from a NOESY correlation between H-15 and H-12 β (δ_{H} 1.92, m). The NOESY spectrum showed correlations of Me-18 (δ_{H} 0.97, s) with H-5 (δ_{H} 1.31) and H-6 (δ_{H} 4.34), indicating the proximity of these protons. Since Me-18 and H-5 in labdanes are α -oriented, H-6 will be α -equatorial. Consequently, the 6-OH has a β -axial orientation. Based on these data, **2** was identified as 9,13:15,16-diepoxyabdane-6 β ,15 α -diol.

Leoleorin F (**3**) was isolated as a colorless oil, $[\alpha]_{\text{D}}^{23} -21.6$, and its molecular formula was determined to be C₂₀H₃₄O₄ by HR-ESIMS (m/z 361.2357 [M + Na]⁺). The ¹H and ¹³C NMR (Tables 1 and 2), and HSQC data confirmed the presence of three tertiary methyl groups [δ_{H} 1.24 (s), 1.20 (s), and 0.97 (s)], one methyl doublet at δ_{H} 0.88 (d, $J = 6.8$ Hz), two oxygenated quaternary carbons (δ_{C} 94.3, 90.6), one oxymethylene group [δ_{C} 64.9; δ_{H} 4.09 (m), 3.77 (m)], and a hemiacetal group [δ_{C} 98.8; δ_{H} 4.70 (s)]. With four degrees of unsaturation and the absence of sp² or sp carbon resonances and UV absorption, compound **3** should be a labdane diterpenoid with a spirotetrahydrofuran structural moiety. The NMR spectroscopic data are similar to those of 9,13:15,16-diepoxyabdane-6 β ,16-diol (Naidoo et al., 2011), which has been identified as an α , β anomeric mixture at C-16 with the configuration of C-13 undetermined. In the NOESY spectrum (Fig. 4), NOEs were detected between Me-18 (δ_{H} 0.97) and H-5 (δ_{H} 1.33), Me-18 and H-6 (δ_{H} 4.34), Me-20 (δ_{H} 1.24) and H-11 (δ_{H} 2.14, 2.09), Me-20 and H-8 (δ_{H} 2.11) establishing the relative configurations at C-5, C-6, C-8, C-9, and C-10. Similarly to compound **2**, the configuration at C-13 was determined as *S* from the NOE correlations between H-1 α (δ_{H} 1.47) and H-16 (δ_{H} 4.70), between H-14 (δ_{H} 2.35) and Me-17 (δ_{H} 0.88), and the latter also confirmed the α -orientation of Me-17. Therefore, the structure of **3** was identified as 9,13:15,16-diepoxyabdane-6 β , 16 β -diol.

16-*epi*-Leoleorin F (**4**), was isolated as a white powder, and its molecular formula C₂₀H₃₄O₄ was determined to be same as that of **3** by HR-ESIMS (m/z 361.2359 [M + Na]⁺). The ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2) were very close to those of **3**. The major difference between **3** and **4** is the opposite sign of their specific rotation ($[\alpha]_{\text{D}}^{23} +30.0$ for **4**, and -21.6 for **3**), and the opposite configuration of C-16 in **4** as indicated by NOESY correlations (Fig. 5). Thus **4** was identified as a C-16 epimer of **3**, 9,13:15,16-diepoxyabdane-6 β ,16 α -diol.

Leoleorin G (**5**) was obtained as a colorless oil, $[\alpha]_{\text{D}}^{23} -20.5$, and its molecular formula was determined as C₂₀H₂₈O₄ by HR-ESIMS (m/z 355.1889 [M + Na]⁺). Its UV maximum absorbance was at 233 nm. The ¹³C NMR and DEPT spectra (Table 2) showed 20 carbon resonances indicating four methyl, seven methylene (one oxymethylene at δ_{C} 77.8), two methine (including one olefinic carbon), and seven quaternary carbons. Analysis of the NMR spectroscopic data (Tables 1 and 2) established the characteristic features of an α , β -unsaturated carbonyl moiety [δ_{C} 172.6 (C-5), 123.0 (C-6), 199.2 (C-7); δ_{H} 5.93 (s, H-6)] analogous to that present in compound Y, which was previously isolated from the same species (Kaplan and Rivett, 1968). Additionally, the HMBC spectrum showed correlations of H₂-16 [δ_{H} 4.14 (d, $J = 8.8$), 3.99 (d, $J = 8.8$)] and H₂-14 [δ_{H} 2.80 (d, $J = 17.2$), 2.43 (d, J

= 17.2)] with the carbonyl carbon at δ_C 174.2, indicating the presence of a five-membered lactone ring. Thus structure **5** was determined to be 9,13-epoxylabd-5-en-7-on-15,16-olide.

Leoleorin H (**6**), was isolated as a white powder ($[\alpha]_D^{23} -29.5$) that gave an $[M + H]^+$ ion at m/z 379.2468 in HR-ESIMS, consistent with a molecular formula of $C_{22}H_{34}O_5$, requiring six degrees of unsaturation. The similarity of the UV spectra of **6** and **5** suggested the presence of an α,β -unsaturated carbonyl moiety in **6**, which was also consistent with the NMR spectroscopic data (Tables 1 and 2) of the conjugated carbonyl unit [δ_C 172.3 (C-5), 122.9 (C-6), 199.9 (C-7); δ_H 5.90 (H-6, s)]. Twenty two resonances ($7 \times C$; $2 \times CH$; $8 \times CH_2$; $5 \times CH_3$) were observed in the ^{13}C NMR and DEPT spectra (Table 2). The signals of an acetoxy group [δ_C 171.1 ($CH_3C=O$), 20.9 ($CH_3C=O$); δ_H 1.90 (3H, s)] were observed. The HMBC correlation between the acetate carbonyl carbon (δ_C 171.1) and oxymethylene protons [δ_H 3.93 (2H, m), H₂-15], and the absence of HMBC correlation between the oxymethylene group at δ_H 3.29 (1H, d, $J = 11.2$), 3.38 (1H, d, $J = 11.2$) and the acetoxy carbonyl carbon (δ_C 171.1), showed a seco-type arrangement similar to compound **1**, in which the hydroxy group at C-15 was acetylated. Thus, **6** was identified as 15-acetoxy-9,13-epoxy-16-hydroxylabd-5-en-7-one.

Leoleorin I (**7**), was obtained as a colorless oil, $[\alpha]_D^{23} -36.5$, and its molecular formula was determined as $C_{20}H_{32}O_4$ by HR-ESIMS (m/z 359.2208 $[M + Na]^+$). The 1H and ^{13}C NMR spectroscopic data (Tables 1 and 2), DEPT, HMBC, HMQC and specific rotation of **7** and **6** were similar, except for the absence of resonances for the acetyl group in **7**. Thus, **7** was identified as 9,13-epoxy-15,16-dihydroxylabd-5-en-7-one.

Leoleorin J (**8**), was obtained as a colorless oil, $[\alpha]_D^{23} -69.6$, and its molecular formula was determined as $C_{20}H_{34}O_4$ by HR-ESIMS (m/z 361.2359 $[M + Na]^+$). The 1H and ^{13}C NMR (Tables 1 and 2), and UV spectra showed that the conjugated carbonyl moiety in **7** was replaced by an allylic alcohol functionality [δ_H 5.57 (d, $J = 1.6$ Hz, H-6), 3.95 (dd, $J = 9.2$, 1.6 Hz, H-7); δ_C 150.2 (C-5), 122.5 (C-6), 72.1 (C-7)] in the structure of **8**. Judging from the molecular formula and putative bio synthetic origin, **8** may be produced by enzymatic reduction of **7** to the corresponding allylic alcohol derivative, or obtained from **1** by dehydration followed by an allylic oxidation. The β -orientation of the 7-OH group was deduced from the NOESY correlation between H-7 (δ_H 3.95, 1H, dd, $J = 9.2$, 1.6) and Me-17 (δ_H 1.12, 3H, d, $J = 6.8$). Thus, **8** was identified as 9,13-epoxylabd-5-ene-7 β ,15,16-triol.

The remaining three compounds **9–11** were earlier reported as compound Y (Kaplan and Rivett, 1968), 9,13-epoxy-6-hydroxylabd-15,16-olide (Naidoo et al., 2011), and semi-synthetic derivative anhydro-Y (Cragg and Little, 1962; Kaplan and Rivett, 1968). Because of their structural similarity to compounds **1–8**, renaming of their names to leoleorins A-C is proposed. Leoleorin A (**9**) was obtained as white crystals, $[\alpha]_D^{23} -22.5$, and its ESIMS spectrum gave an ion peak at m/z 317.2 ($[M + H]^+$). Since leoleorins A (**9**) and B (**10**) were reported (Cragg and Little, 1962; Kaplan and Rivett, 1968) before the advent of high resolution NMR instruments their 1H and ^{13}C NMR assignments are presented here. The 1H NMR spectrum of **9** (Table 1) exhibited the typical proton resonances of a β -substituted furan moiety at δ_H 7.35, 7.23, and 6.28, three methyl singlets at δ_H 1.36, 1.23, and 1.18, and one methyl doublet at δ_H 1.28 ($J = 6.8$ Hz), coupled with a vicinal proton (2.78, q, $J = 6.8$ Hz). The signals of an α,β -unsaturated carbonyl moiety were observed at δ_H 6.12 (s, H-6), and δ_C 175.3 (C-5), 123.7 (C-6), 200.1 (C-7), which is in agreement with the UV absorption at 240 nm. The structure and absolute configuration of **9** were unambiguously defined by single-crystal X-ray crystallography as (8*S*,9*R*,10*S*)-15,16-epoxy-9 α -hydroxylabda-5,13(16),14-trien-7-one (Fig. 6)(Kaplan and Rivett, 1968).

Leoleorin C (**10**) was isolated as a yellow oil, $[\alpha]_D^{23} +23.9$. Comparison of the NMR spectroscopic data of **10** with those of **9** showed two additional sp^2 carbon signals at δ_C 130.8 and 163.1, and replacement of the Me-17 doublet with singlet (δ_H 1.95) in the spectrum of compound **10**. These features suggested that **10** might derive from **9** via dehydration with formation of C-8 – C-9 double bond. Based on these data, compound **10** was identified as 15,16-epoxylabda-5,8,13(16),14-tetraen-7-one, which is identical with anhydro-Y obtained semi-synthetically from compound Y (Kaplan and Rivett, 1968).

2.2 Binding affinities of isolated compounds to CNS receptors

Compounds (**1** – **11**) were screened against a battery of 26 G-protein-coupled receptors including serotonin, adrenergic, dopamine, histamine, muscarinic acetylcholine, and opioid receptors. Additionally, the compounds were screened against biogenic amine transporters and the sigma1 and sigma2 receptors. The data (Table 3) demonstrate that some of the compounds displayed activity in several assays. Compounds **1**, **6**, **7**, and **9** inhibited radioligand binding at the serotonin 5-HT_{1A} receptor (inhibition: 62.3, 50.0, 61.9, and 60.4%, respectively), while compound **8** showed 57.0% inhibition at the 5HT₃ receptor. Compounds **1**, **2**, **5**, and **6** showed activity at the D1-dopamine receptor (inhibition: 50.3, 57.4, 62.3, and 55.7 %, respectively). Compounds **2**, **3**, **10**, and **11** showed significant activity at the H1-histamine receptor (inhibition: 54.8, 56.3, 52.3, and 63.2%, respectively). In addition, compounds **4** and **11** inhibited binding at the M3-acetylcholine, and sigma-1 receptors (inhibition: 55.1, and 59.1 %, respectively). In the secondary assay of competitive binding compound **11** showed moderate affinity ($K_i = 2.9 \mu M$ as compare to positive control, haloperidol $K_i = 7$ nM) for the Sigma 1 receptor. In all other assays, the compounds displayed K_i values > 10,000 nM and were not pursued further.

2.3 Concluding remarks

Eight labdane diterpenoids (**1**–**8**) together with three known compounds (**9**–**11**), were isolated from leaves of *L. leonurus*. Their structures were established on the basis of spectroscopic methods as well as X-ray crystallographic analyses. In the radioligand binding assay, leoleorin C (**11**) showed moderate binding affinity ($K_i = 2.9 \mu M$) to the Sigma 1 receptor, which could be responsible for the psychoactive properties of this plant.

Many labdane diterpenoids were previously isolated from *L. leonurus* as well as other *Leonotis* species, and they may therefore serve as chemical markers for characterization of the *Leonotis* genus. In the current study, compounds **1**–**8** were obtained as new compounds in relatively high concentrations; their presence being overlooked in previous investigations is presumably attributable to variations of harvest time, geographical, climate, and environmental factors.

3. Experimental

3.1 General Experimental Procedures

Melting points were measured on OptiMelt MPA100 Automated Melting Point System and are uncorrected. Optical rotations were acquired with a Rudolph Research Analytical Autopol IV automatic polarimeter. IR spectra were recorded on a Bruker Tensor 27 spectrophotometer. NMR spectra were acquired on a Bruker DRX NMR spectrometer operating at 400 MHz for ¹H and 100 MHz for ¹³C with a 3 mm direct carbon probe. The mass detector was time of flight (model G1969A) equipped with an electrospray ionization interface and was controlled by Aligent software (Aligent Mass Hunter Work Station, A. 02.01).

3.2 Plant Material

Leafy freeze-dried material of *L. leonurus* was purchased from IAmShaman Shop (P.O. Box 12618 Chicago, IL 60612) in June 2010, and authenticated by one of the co-authors (Charles Burandt Jr.). A voucher specimen (Code: hkwu-LL-20101006) was deposited at the Department of Pharmacognosy, School of Pharmacy, University of Mississippi.

3.3 Extraction and Isolation

The dried and powdered leaves of *L. leonurus* (411 g) were extracted with acetone (3×2.0 L; each 24 h) by percolation at room temperature. The solvent was evaporated under reduced pressure at 40 °C to yield 42 g (10.2 %) of a viscous syrup, which was dissolved in CH₂Cl₂:MeOH (60 mL, 1:1, v/v) and mixed with silica gel (85 g, 200–300 mesh), and dried at room temperature in a rotary evaporator under reduced pressure to give a fine powder. The latter was separated by silica gel column chromatography (CC) (200 g), eluted with hexanes-EtOAc (10:1, 4:1, 2:1, 1:1, 1:4, and 0:1, v/v) to give six sub-fractions A - F. Fr. A (3.1 g) containing fatty acids and pigments, was not studied further. Fr. B (9.5 g) was purified by Sephadex LH-20 (CH₂Cl₂-MeOH 1:1), and then silica gel CC (100 g), eluted with hexanes-EtOAc (9:1, 7:1, 5:1, 3:1, v/v) to yield **9** (1 g), **3** (1 g), **11** (22 mg) and **10** (40 mg), respectively. Fr. C (2.8 g) was purified by silica gel CC (100 g), eluted with hexanes-EtOAc (5:1, 4:1, 3:1, 2:1, v/v) to yield **4** (630 mg) and **2** (100 mg). Fr. D (5 g) was purified by silica gel CC (100 g), eluted with hexanes-EtOAc (3:1, 2:1, 1:1, v/v) to yield **5** (500 mg) and **6** (200 mg). Fr. E (5.0 g) was purified by silica gel CC (160 g), eluted with hexanes-EtOAc (3:1, 2:1, 1:1, v/v) to yield **1** (300 mg). Fr. F (2 g) was purified by Sephadex LH-20 (CH₂Cl₂-MeOH 1:1) and then silica gel CC (160 g), eluted with EtOAc to yield **7** (500 mg) and **8** (20 mg).

3.4 Characterization of compounds

Leoleorin A [(8S,9R,10S)-15,16-epoxy-9 α -hydroxylabda-5,13(16), 14-trien-7-

one, 9]—white crystals (from EtOAc), mp 194–197 °C; $[\alpha]_D^{23}$ –22.5 (*c* 0.08, MeOH); UV (MeOH) λ_{\max} (log ϵ) 240 (4.17) nm; IR (KBr) ν_{\max} 3612, 2943, 1654, 1519, 1463 cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 317.2 [M + H]⁺.

Leoleorin B (15,16-epoxylabda-5,8,13(16),14-tetraen-7-one, 10)—yellow oil; $[\alpha]_D^{23}$ +23.9 (*c* 0.1, MeOH); UV (MeOH) λ_{\max} (log ϵ) 245 (3.98) nm; IR (KBr) ν_{\max} 2944, 1651, 1622, 1465, 1364 cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 299.2 [M + H]⁺.

Leoleorin C (9,13-epoxy-6-hydroxylabdan-15,16-olide, 11)—white powder; $[\alpha]_D^{23}$ –2.1 (*c* 0.8, MeOH); ESIMS *m/z* 695.5 [2M + Na]⁺; ¹H and ¹³C NMR spectroscopic data were identical with 9,13-epoxy-6-hydroxylabdan-16,15-olide (Naidoo et al., 2011).

Leoleorin D[(5S,6R,8R,9R,10S,13S)-9,13-epoxylabdane-6,15,16-triol, 1]—white crystals (from EtOAc), mp 116–117 °C; $[\alpha]_D^{23}$ –7.5 (*c* 1.1, MeOH); for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS *m/z* 363.2499 [M + Na]⁺ (calcd for C₂₀H₃₆O₄Na, 363.2511).

Leoleorin E (9,13:15,16-diepoxyabdane-6 β ,15 α -diol, 2)—colorless oil; $[\alpha]_D^{23}$ –3.3 (*c* 0.06, MeOH); for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS *m/z* 361.2351 [M + Na]⁺ (calcd for C₂₀H₃₄O₄Na, 361.2355).

Leoleorin F (9,13:15,16-diepoxyabdane-6 β ,16 β -diol, 3)—colorless oil; $[\alpha]_D^{23}$ -21.6 (c 0.04, MeOH); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS m/z 361.2357 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{34}\text{O}_4\text{Na}$, 361.2355).

16-epi-Leoleorin F (9,13:15,16-diepoxyabdane-6 β ,16 α -diol, 4)—white powder; $[\alpha]_D^{23}$ $+30.0$ (c 0.2, MeOH); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS m/z 361.2359 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{34}\text{O}_4\text{Na}$, 361.2355).

Leoleorin G (9,13-epoxyabd-5-en-7-on-15,16-olide, 5)—colorless oil; $[\alpha]_D^{23}$ -20.5 (c 0.4, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 233 (4.06) nm; IR (KBr) ν_{max} 2930, 1783, 1667, 1167, 1025 cm^{-1} ; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS m/z 355.1889 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{28}\text{O}_4\text{Na}$, 355.1885).

Leoleorin H (15-acetoxy-9,13-epoxy-16-hydroxyabd-5-en-7-one, 6)—white powder; $[\alpha]_D^{25}$ -29.5 (c 0.4, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 234 (4.15) nm; IR (KBr) ν_{max} 3462, 2930, 1736, 1665, 1466, 1367, 1241 cm^{-1} ; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS m/z 379.2468 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{22}\text{H}_{35}\text{O}_5$, 379.2484).

Leoleorin I (9,13-epoxy-15,16-dihydroxyabd-5-en-7-one, 7)—colorless oil; $[\alpha]_D^{23}$ -36.5 (c 0.06, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 240 (4.16) nm; IR (KBr) ν_{max} 3368, 2929, 1660, 1465, 1040, 985 cm^{-1} ; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS m/z 359.2208 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_4\text{Na}$, 359.2198).

Leoleorin J (9,13-epoxyabd-5-ene-7 β ,15,16-triol, 8)—colorless oil; $[\alpha]_D^{23}$ -69.6 (c 0.02, MeOH); IR (KBr) ν_{max} 3565, 1650, 1041, 1024 cm^{-1} ; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS m/z 361.2359 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{34}\text{O}_4\text{Na}$, 361.2355).

3.5 X-ray Crystallography

The crystal structures and absolute configuration of **1** and **9** were determined using data collected at $T = 90$ K with $\text{CuK}\alpha$ radiation ($\lambda = 1.54178$ Å) on a Bruker APEX-II CCD diffractometer, equipped with an Oxford Cryostream cooler. Structures were solved using the program SHELXS-97 and refined anisotropically by full-matrix least squares on F^2 using SHELXL-97 (Sheldrick, 2008). OH hydrogen coordinates were refined; other H atoms were visible in difference maps, but were placed in idealized positions for the refinements. The absolute configurations were determined by refinement of the Flack (Flack, 1983) parameter based on resonant scattering of the light atoms and computation of the Hooft parameter (Hooft et al., 2008), in both cases yielding a probability of 1.000 that the reported configuration is correct. Crystal data: leoleorin D (**1**), $\text{C}_{20}\text{H}_{36}\text{O}_4$, $M_r = 340.49$, orthorhombic space group $\text{P}2_12_12_1$, $a = 6.9830$ (3), $b = 13.9703$ (6), $c = 19.1528$ (8) Å, $V = 1868.44$ (14) Å³, $Z = 4$, $D_x = 1.210$ Mg m⁻³, $\theta_{\text{max}} = 69.4^\circ$, $R = 0.027$ for 3472 data and 231 refined parameters. The Flack parameter is 0.08 (13) and the Hooft parameter is 0.04 (3) for 1452 Bijvoet pairs (98% coverage); leoleorin A (**9**), $\text{C}_{20}\text{H}_{28}\text{O}_3$, $M_r = 316.42$, orthorhombic space group $\text{P}2_12_12_1$, $a = 6.5463$ (2), $b = 11.4968$ (4), $c = 22.8194$ (9) Å, $V = 1717.42$ (10) Å³, $Z = 4$, $D_x = 1.224$ Mg m⁻³, $\theta_{\text{max}} = 67.6^\circ$, $R = 0.030$ for 3061 data and 216 refined parameters. The Flack parameter is 0.05 (18) and the Hooft parameter is 0.00 (9) for 1265 Bijvoet pairs (98% coverage). The crystal structures have been deposited with the Cambridge Crystallographic Data Centre, deposition numbers CCDC 86124 for leoleorin D (**1**) and CCDC 861242 for leoleorin A (**9**).

3.6 Radioligand Binding Assays

Receptor binding profiles were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth M.D., Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. For initial screening, compounds were tested at concentrations of 10 $\mu\text{mol/L}$. Detailed receptor-binding protocols are available at their web site (<http://pdsp.med.unc.edu>).

Highlights

► Eleven labdane diterpenoids were isolated from the leaves of *Leonotis leonurus*. ► Eight compounds were hitherto unknown. ► All compounds were evaluated for their binding affinities to various CNS receptors. ► Leoleorin C (**11**) showed weak binding affinity ($K_i = 2.9 \mu\text{M}$) to the Sigma 1 receptor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project was supported by a grant from National Institutes of Health (R03DA023491). We are grateful to Ms. Bin Wang at the Department of Pharmacognosy for assistance with the HRESIMS.

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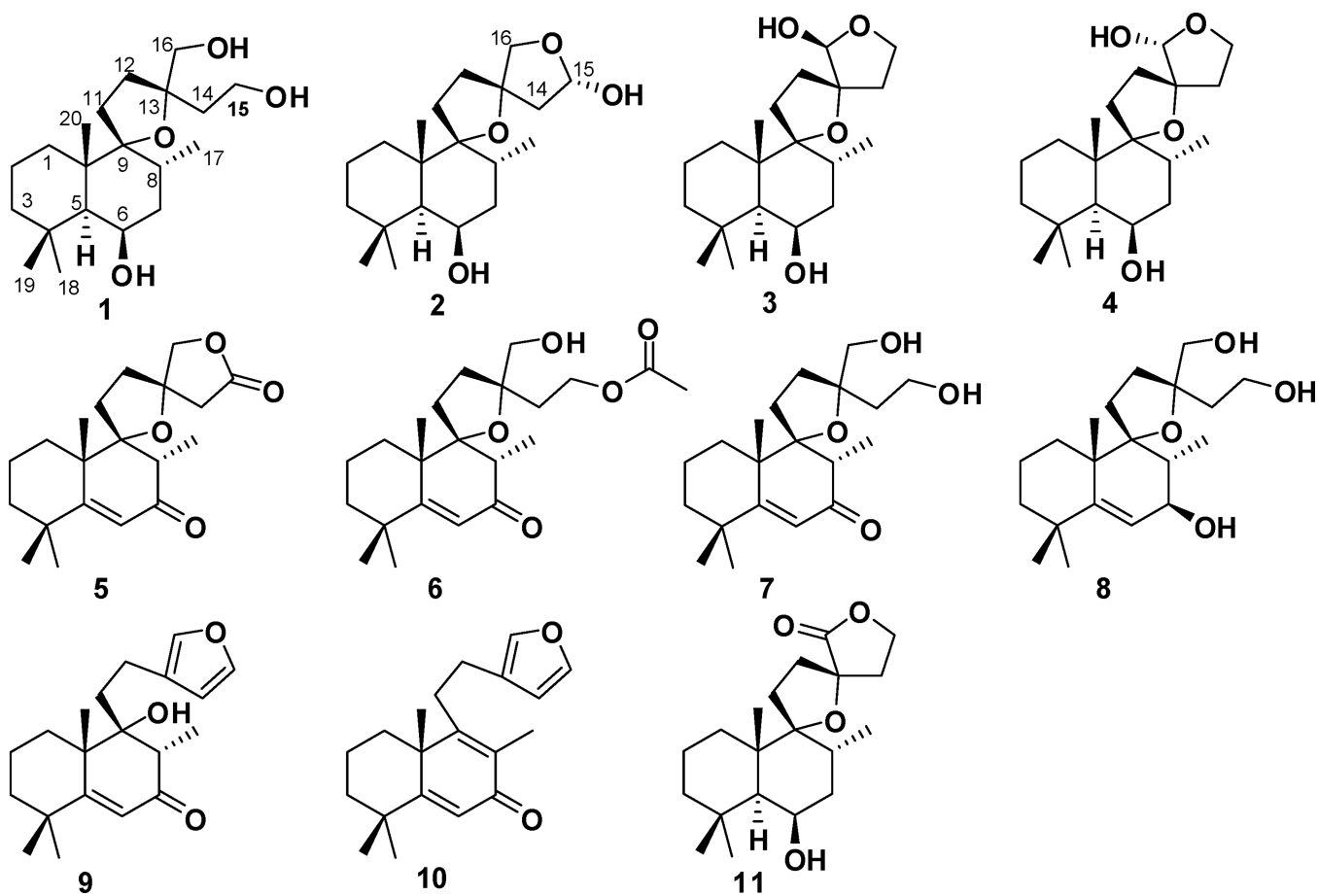


Figure 1.
Structures of compounds 1–11.

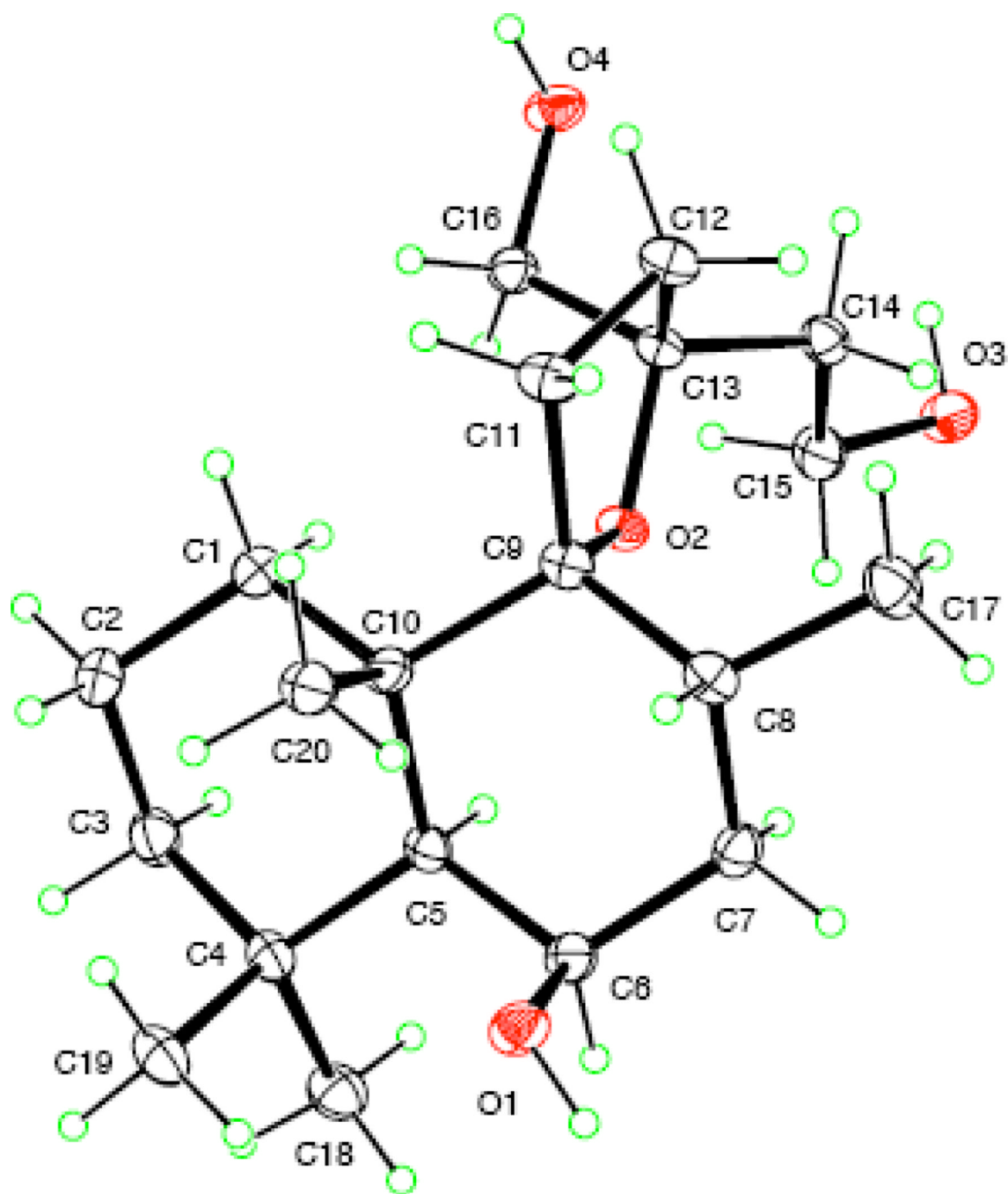


Figure 2.
ORTEP drawing of 1.

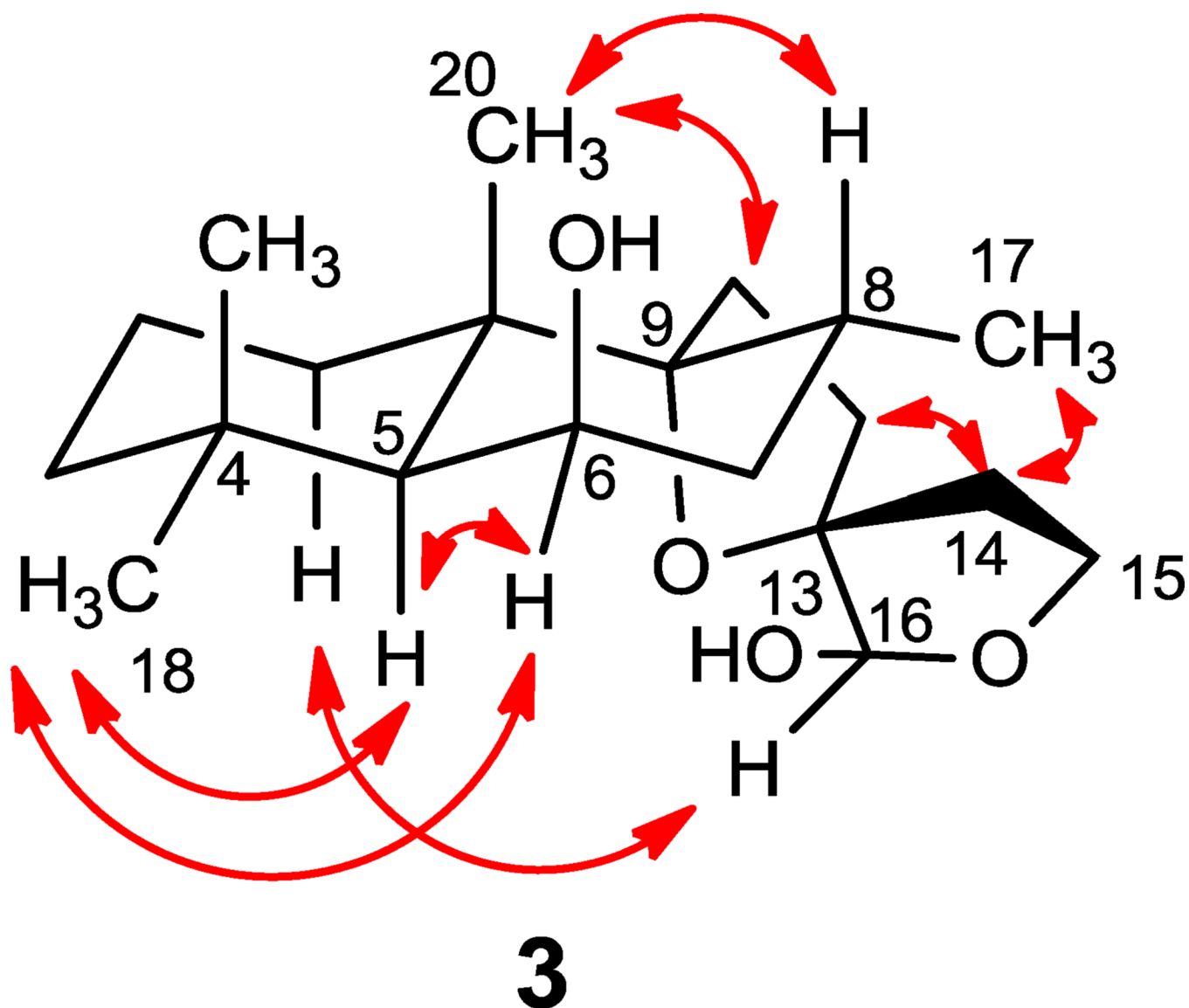


Figure 4.
Key NOESY correlations of **3**.

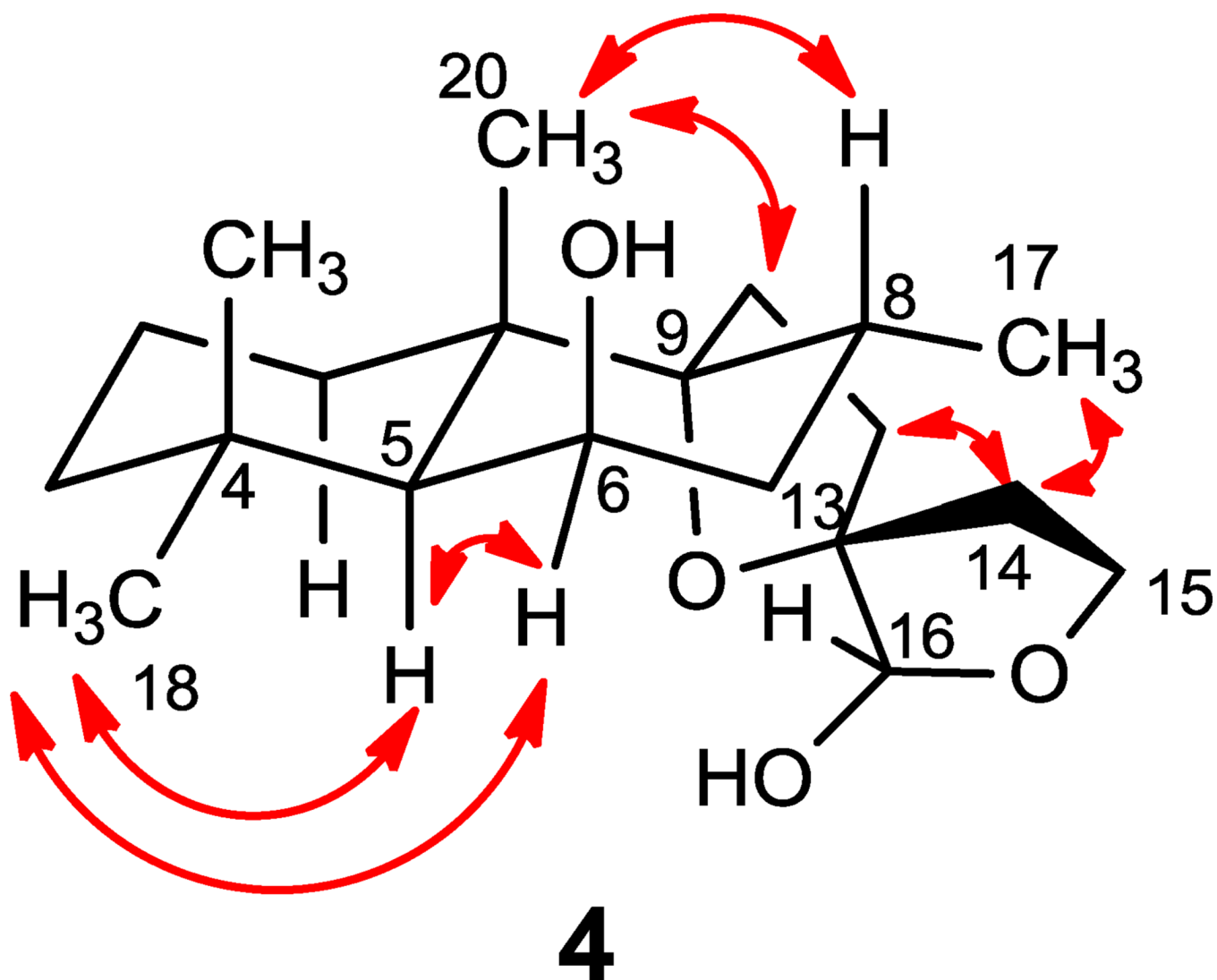


Figure 5.
Key NOESY correlations of **4**.

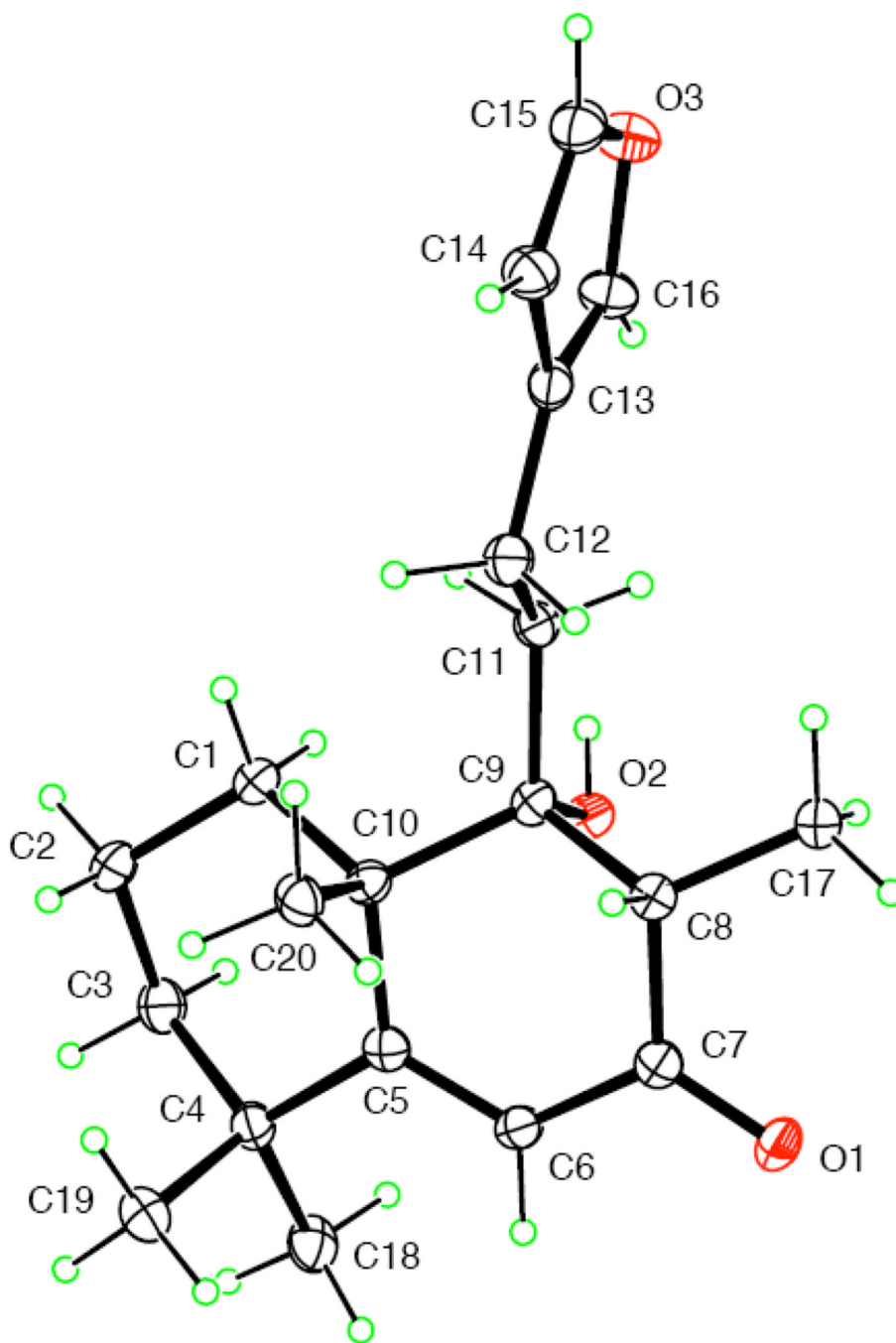


Figure 6.
ORTEP drawing of **9**.

Table 1

¹H NMR data of compounds 1–10 (CDCl₃, δ ppm, *J* in Hz, 400 MHz)

| Proton | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--------|----------------|---------------|---------------|---------------|----------------|----------------|----------------|--------------------|---------------|---------|
| 1 | 1.75, m | 1.89, m | 1.47, m | 1.46, m | 1.54, m | 1.54, m | 1.84, m | 1.63, m | 1.98, m | 2.13, m |
| 2 | 1.40, m | 1.42, m | 1.29, m | 1.33, m | 1.44, m | 1.45, m | 1.67, m | 1.51, m | 1.88, m | 1.40, m |
| 3 | 1.67, m | 1.66, m | 1.62, m | 1.65, m | 1.78, m | 1.73, m | 1.73, m | 1.80, m | 1.82, m | 1.92, m |
| 4 | 1.49, m | 1.45, m | 1.45, m | 1.48, m | 1.61, m | 1.59, m | 1.57, m | 1.49, m | 1.67, m | 1.66, m |
| 5 | 1.35, m | 1.32, m | 1.28, m | 1.32, m | 1.50, m | 1.45, m | 1.80, m | 1.65, m | 1.55, m | 1.64, m |
| 6 | 1.15, m | 1.17, m | 1.16, m | 1.16, m | 1.34, m | 1.34, m | | 1.59, m | 1.43, m | 1.35, m |
| 7 | 1.29, br s | 1.31, s | 1.33, d (1.5) | 1.34, br s | | | | | | |
| 8 | 4.36, br s | 4.34, m | 4.34, m | 4.36, m | 5.93, s | 5.90, s | 5.89, s | 5.57, d (1.6) | 6.12, s | 6.29, s |
| 9 | 1.72, m | 1.65, m | 1.74, m | 1.75, m | | | | 3.95, dd (1.6,9.2) | | |
| 10 | 1.43, m | 1.46, m | 1.43, m | 1.45, m | | | | | | |
| 11 | 2.10, m | 2.09, m | 2.11, m | 2.25, m | 2.80, q (6.8) | 2.85, q(6.8) | 2.87, q (6.8) | 1.93, m | 2.78, q (6.8) | |
| 12 | 2.09, m | 2.10, m | 2.14, m | 2.14, m | 2.22, m | 2.05, m | 2.06, m | 2.04, m | 1.99, m | 2.65, m |
| 13 | 1.70, m | 1.76, m | 2.09, m | 1.79, m | 1.92, m | 1.55, m | 1.78, m | 1.83, m | 1.71, m | 2.50, m |
| 14 | 2.13, m | 2.06, m | 2.36, m | 2.35, m | 2.10, m | 1.97, m | 1.83, m | 1.84, m | 2.62, m | 2.59, m |
| 15 | 1.70, m | 1.92, m | 1.93, m | 1.94, m | 2.05, m | 1.85, m | 1.68, m | 1.36, m | 2.53, m | 2.53, m |
| 16 | 2.05, m | 2.28, m | 2.35, m | 2.37, m | 2.80, d (17.2) | 2.10, m | 1.80, m | 2.05, m | 6.28, s | 6.32, s |
| 17 | 1.95, m | 2.09, m | 1.93, m | 1.88, m | 2.43, d (17.2) | 1.78, m | | 1.83, m | | |
| 18 | 3.85, m | 5.41, d (4.8) | 4.09, m | 4.10, m | | 4.04, m | 3.53, m | 3.78, m | 7.35, s | 7.37, s |
| 19 | 3.62, d (10.8) | 4.27, d (8.8) | 3.77, m | 3.79, m | | 3.93, m | | | | |
| 20 | 3.50, d (10.8) | 3.55, d (8.8) | 4.70, s | 4.72, s | 4.14, d (8.8) | 3.38, d (11.2) | 3.41, d (11.2) | 3.57, d (10.8) | 7.23, s | 7.28, s |
| 21 | 0.93, d (6.8) | 0.92, d (6.8) | 0.88, d (6.8) | 0.89, d (6.8) | 3.99, d (8.8) | 3.29, d (11.2) | 3.32, d (11.2) | 3.48, d (10.8) | | |
| 22 | 1.01, s | 0.97, s | 0.97, s | 0.99, s | 1.08, s | 1.06, s | 1.06, s | 1.12, d (6.8) | 1.28, d (6.8) | 1.95, s |
| 23 | 1.24, s | 1.22, s | 1.20, s | 1.11, s | 1.11, s | 1.08, s | 1.08, s | 1.09, s | 1.18, s | 1.21, s |
| 24 | 1.27, s | 1.24, s | 1.24, s | 1.26, s | 1.27, s | 1.25, s | 1.26, s | 1.14, s | 1.23, s | 1.32, s |
| 25 | | | | | | | | 1.20, s | 1.36, s | 1.33, s |
| 26 | | | | | | 1.90, s | | | | |

Table 2

¹³C NMR data of compounds **1** – **10** (CDCl₃, δ ppm, 100 MHz)

| Carbon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1 | 34.3 <i>t</i> | 34.3 <i>t</i> | 33.2 <i>t</i> | 33.2 <i>t</i> | 30.5 <i>t</i> | 30.6 <i>t</i> | 30.5 <i>t</i> | 31.1 <i>t</i> | 30.8 <i>t</i> | 34.6 <i>t</i> |
| 2 | 18.9 <i>t</i> | 18.8 <i>t</i> | 18.7 <i>t</i> | 18.7 <i>t</i> | 17.6 <i>t</i> | 17.7 <i>t</i> | 17.8 <i>t</i> | 18.3 <i>t</i> | 17.5 <i>t</i> | 18.2 <i>t</i> |
| 3 | 44.3 <i>t</i> | 44.0 <i>t</i> | 44.1 <i>t</i> | 44.1 <i>t</i> | 39.0 <i>t</i> | 39.1 <i>t</i> | 39.1 <i>t</i> | 40.6 <i>t</i> | 38.7 <i>t</i> | 40.5 <i>t</i> |
| 4 | 34.5 <i>s</i> | 34.4 <i>s</i> | 34.3 <i>s</i> | 34.3 <i>s</i> | 36.9 <i>s</i> | 37.0 <i>s</i> | 37.0 <i>s</i> | 35.9 <i>s</i> | 36.8 <i>s</i> | 37.3 <i>s</i> |
| 5 | 50.5 <i>d</i> | 50.0 <i>d</i> | 50.0 <i>d</i> | 50.0 <i>d</i> | 172.6 <i>s</i> | 172.3 <i>s</i> | 173.0 <i>s</i> | 150.2 <i>s</i> | 175.3 <i>s</i> | 172.3 <i>s</i> |
| 6 | 68.0 <i>d</i> | 67.8 <i>d</i> | 67.8 <i>d</i> | 67.9 <i>d</i> | 123.0 <i>d</i> | 122.9 <i>d</i> | 122.7 <i>d</i> | 122.5 <i>d</i> | 123.7 <i>d</i> | 123.7 <i>d</i> |
| 7 | 40.7 <i>t</i> | 41.0 <i>t</i> | 40.7 <i>t</i> | 40.7 <i>t</i> | 199.2 <i>s</i> | 199.9 <i>s</i> | 200.5 <i>s</i> | 72.1 <i>d</i> | 200.1 <i>s</i> | 187.0 <i>s</i> |
| 8 | 30.9 <i>d</i> | 30.6 <i>d</i> | 30.8 <i>d</i> | 30.8 <i>d</i> | 47.1 <i>d</i> | 47.6 <i>d</i> | 47.6 <i>d</i> | 41.5 <i>d</i> | 47.8 <i>d</i> | 130.8 <i>s</i> |
| 9 | 94.1 <i>s</i> | 94.5 <i>s</i> | 94.3 <i>s</i> | 94.3 <i>s</i> | 96.1 <i>s</i> | 95.5 <i>s</i> | 95.5 <i>s</i> | 94.4 <i>s</i> | 79.2 <i>s</i> | 163.1 <i>s</i> |
| 10 | 42.9 <i>s</i> | 42.7 <i>s</i> | 42.8 <i>s</i> | 42.8 <i>s</i> | 44.7 <i>s</i> | 45.0 <i>s</i> | 45.1 <i>s</i> | 43.5 <i>s</i> | 46.5 <i>s</i> | 44.3 <i>s</i> |
| 11 | 28.2 <i>t</i> | 29.3 <i>t</i> | 29.2 <i>t</i> | 29.2 <i>t</i> | 29.8 <i>t</i> | 29.6 <i>t</i> | 29.3 <i>t</i> | 29.3 <i>t</i> | 34.3 <i>t</i> | 31.0 <i>t</i> |
| 12 | 35.7 <i>t</i> | 34.5 <i>t</i> | 36.4 <i>t</i> | 36.4 <i>t</i> | 37.6 <i>t</i> | 35.6 <i>t</i> | 34.9 <i>t</i> | 35.1 <i>t</i> | 21.1 <i>t</i> | 24.1 <i>t</i> |
| 13 | 85.3 <i>s</i> | 89.8 <i>s</i> | 90.6 <i>s</i> | 90.6 <i>s</i> | 86.2 <i>s</i> | 85.2 <i>s</i> | 86.0 <i>s</i> | 85.5 <i>s</i> | 125.1 <i>s</i> | 124.4 <i>s</i> |
| 14 | 28.9 <i>t</i> | 46.0 <i>t</i> | 35.2 <i>t</i> | 35.6 <i>t</i> | 41.9 <i>t</i> | 34.3 <i>t</i> | 40.3 <i>t</i> | 40.3 <i>t</i> | 110.7 <i>d</i> | 110.6 <i>d</i> |
| 15 | 59.6 <i>t</i> | 98.9 <i>t</i> | 64.9 <i>t</i> | 65.0 <i>t</i> | 174.2 <i>s</i> | 61.4 <i>t</i> | 58.7 <i>t</i> | 59.5 <i>t</i> | 142.9 <i>d</i> | 143.1 <i>d</i> |
| 16 | 66.8 <i>t</i> | 76.8 <i>d</i> | 98.8 <i>d</i> | 98.8 <i>d</i> | 77.8 <i>t</i> | 65.6 <i>t</i> | 66.3 <i>t</i> | 66.6 <i>t</i> | 138.5 <i>d</i> | 138.6 <i>d</i> |
| 17 | 18.0 <i>q</i> | 17.8 <i>q</i> | 17.1 <i>q</i> | 17.1 <i>q</i> | 9.5 <i>q</i> | 9.7 <i>q</i> | 9.8 <i>q</i> | 13.2 <i>q</i> | 9.1 <i>q</i> | 11.3 <i>q</i> |
| 18 | 33.3 <i>q</i> | 33.3 <i>q</i> | 33.3 <i>q</i> | 33.3 <i>q</i> | 31.4 <i>q</i> | 31.5 <i>q</i> | 31.5 <i>q</i> | 32.5 <i>q</i> | 31.5 <i>q</i> | 32.4 <i>q</i> |
| 19 | 24.9 <i>q</i> | 24.8 <i>q</i> | 24.7 <i>q</i> | 24.7 <i>q</i> | 30.7 <i>q</i> | 30.5 <i>q</i> | 30.6 <i>q</i> | 30.7 <i>q</i> | 31.2 <i>q</i> | 28.6 <i>q</i> |
| 20 | 20.6 <i>q</i> | 20.4 <i>q</i> | 20.5 <i>q</i> | 20.5 <i>q</i> | 24.0 <i>q</i> | 23.8 <i>q</i> | 23.7 <i>q</i> | 25.9 <i>q</i> | 24.2 <i>q</i> | 25.4 <i>q</i> |
| 21 | | | | | | 171.1 <i>s</i> | | | | |
| 22 | | | | | | 20.9 <i>q</i> | | | | |

^aMultiplicity has been determined by DEPT experiments and assignments have been made by HSQC and HMBC.

Table 3

Primary CNS receptor binding data of compound 1–11^a

| Compound | Serotonin | | Dopamine | Histamine | Acetylcholine | | Opioid |
|----------|--------------------|-------------------|----------|-----------|---------------|----|--------|
| | 5-HT _{1A} | 5-HT ₃ | D1 | H1 | M3 | M3 | Signal |
| 1 | 62.3 | NA ^b | 50.3 | NA | NA | NA | NA |
| 2 | NA | NA | 57.4 | 54.3 | NA | NA | NA |
| 3 | NA | NA | NA | 56.3 | NA | NA | NA |
| 4 | NA | NA | NA | NA | 55.1 | NA | NA |
| 5 | NA | NA | 62.3 | NA | NA | NA | NA |
| 6 | 50.0 | NA | 55.7 | NA | NA | NA | NA |
| 7 | 61.9 | NA | NA | NA | NA | NA | NA |
| 8 | NA | 57.0 | NA | NA | NA | NA | NA |
| 9 | 60.4 | NA | NA | NA | NA | NA | NA |
| 10 | NA | NA | NA | 52.3 | NA | NA | NA |
| 11 | NA | NA | NA | 63.2 | NA | NA | 59.1 |

^aData represent mean percent inhibition (n = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered >50%. All represent human receptors unless otherwise specified.

^bNA, not active