and then the aqueous layer was concentrated in vacuo. The residue was dissolved in 10 mL of water and 50 mL of ethanol, treated with 2 mL of propylene oxide for 30 min at 50 °C (pH <1 to pH ~2-3), and then concentrated in vacuo. The residue was treated with 50 mL of ethanol, the ethanol was decanted, and then the residue was recrystallized from aqueous ethanol to afford 1.07 g (54%) of 1: mp 245-246 °C (foams); $[\alpha]_D$ = -21.0° (c = 1, 6 N HCl);[9] ^{1}H NMR (D_2O) δ 4.01 (t, J = 7.0 Hz, 1 H), 1.90-2.20 (m, 2 H), 1.60-1.90 (m, 4 H). Anal. Calcd for $C_5H_{12}NO_5P$: C, 30.47; H, 6.14; N, 7.11. Found: C, 30.67; H, 6.07; N, 6.90.

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Determination of a New Sesquiterpene Skeleton through Selective INEPT Spectroscopy[1,2]

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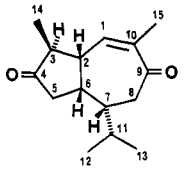
Eupatorium adenophorum Spreng. (Compositae), Saap maa, is native to the Chiang Mai Province of Thailand, and is claimed to be useful in traditional Thai medicine. Recently, the binominal name for this plant has been changed to *Ageratina adenophora* (Spreng.) R. King and H. Robinson.[4] According to *A Geographical Atlas of World Weeds*, the *Ageratina adenophora* (Spreng.) R. King and H. Robinson is synonymous with *Eupatorium adenophorum* Spreng.[5] Previous phytochemical study on this plant reported the isolation[6] of a cadinane-type sesquiterpene 9-oxoageraphorone (**2**), which was also obtained together with its epimer **3** from *Eupatorium trapezoideum* Kunth,[7] subsequently renamed as *Ageratina trapezoidea* (Kunth) R. King and H. Robinson.



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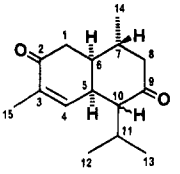

2 10-H α
3 10-H β

We report here on the isolation and structure elucidation of eupatorenone (**1**), the first representative of a new bicyclic sesquiterpene skeleton,[8] which was obtained by chromatographic separation of the petroleum ether soluble part of the ethanolic extract of the whole plant of *Eupatorium adenophorum*. Mass spectrometric analysis of eupatorenone (**1**), mp 66-67 °C, $[\alpha]_D$ +72.2° (MeOH, c 1.3), indicated a molecular ion at m/z 234, corresponding to an elemental composition $C_{15}H_{22}O_2$. Intense absorptions in the UV (MeOH) spectrum at λ_{max} 242 nm (log ε 3.86) and in the IR (KBr) spectrum at ν_{max} 1725, 1710, and 1605 cm^{-1} suggested the presence of both saturated and α,β-unsaturated ketonic groups. The ^{1}H NMR spectrum of eupatorenone, obtained in $CDCl_3$ (Table I), indicated the presence of an olefinic hydrogen (δ 6.35, d, J = 1.5 Hz), both allylic (δ 1.73, d, J = 1.5 Hz) and aliphatic (δ 1.02, d, J = 6.4 Hz) methyl groups, and an isopropyl group (δ 0.88, d, J = 6.1 Hz; 1.08, d, J = 6.1 Hz; 2.00, m). Two geminally coupled methylene groups (δ 2.08, 2.20, J_{gem} = 8.8 Hz; and δ 2.54, 2.81, J_{gem} = 16.6 Hz) were also observed in the molecule.

From the structural elements found, two types of bicyclic sesquiterpene structures could be proposed for eupatorenone; either a condensed cyclopentanone-cycloheptenone structure (**1**) or a cadinene skeleton comprised of a cyclohexanone and a cyclohexenone unit. Two isomeric sesquiterpenes with the latter skeleton, cadinanes **2** and **3**, have already been isolated from *Eupatorium trapezoidum* Kunth (syn. *Adenophora trapezoidea* (Kunth) R. King and H. Robinson), and their structures have been established by a combination of spectroscopic and chemical correlation studies, together with X-ray analysis of a derivative.[8] The IR and ^{1}H and ^{13}C NMR spectral data of eupatorenone differ in numerous ways from the corresponding reported values for cadinanes **2** and **3**. The highest wavenumber for carbonyl absorption in the IR spectrum of **2** and **3** is 1700-1705 cm^{-1} versus 1725 cm^{-1} for eupatorenone. The ^{1}H NMR spectrum in $CDCl_3$ of **3** exhibits three overlapping methyl groups at δ 0.90 and a fourth methyl group at δ 1.60, whereas the corresponding values of eupatorenone are δ 0.88, 1.02, 1.08, and 1.73. In the ^{13}C NMR spectrum of **2**, two doublets and two triplets were reported at δ 45.2, 50.3 and δ 23.3, 33.3, respectively. The ^{13}C NMR spectrum of eupatorenone, however, shows two doublets at δ 28.1 and 39.24 and two triplets at δ 41.04 and 45.80. The reported α_D values for cadinanes **2** and **3** are +156° and +72.2°, respectively, whereas the α_D value of eupatorenone is +72.2°.

On the basis of the above listed spectroscopic differences, we perceived that eupatorenone could not be characterized by a cadinane structure such as as **2** or **3**, where structure elucidation had been performed by reliable chemical derivatization and X-ray crystallography.[7] Therefore, from the structural elements present and the coupling pattern of the homonuclear COSY spectrum (measured either in $CDCl_3$ or in pyridine-d_5), an unsaturated azulene skeleton was suggested for eupatorenone. The presence of a five-membered ring ketone explains the higher wavenumber carbonyl absorption of eupatorenone than that of cadinanes **2** and **3**. Optimum resolution of the ^{1}H NMR signals was achieved in pyridine-d_5 and in $CDCl_3$, with C_6D_6 yielding minimal signal dispersion (Table I). The ^{1}H-^{1}H COSY spectrum indicated a long-range coupling between the allylic methyl protons (δ 1.73) and the vinyl hydrogen 1-H (δ 6.35), which itself was coupled to the anellated methine, 2-H (δ 3.22). From the relatively small (>4 Hz) coupling between 2-H and 6-H (δ 2.28) a cis junction between the five- and seven-membered rings was indicated. An additional small coupling (>1 Hz), observed

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Table I. ^1H and ^{13}C NMR Assignments of Eupatorenone (1)

	$^1H^a$	$^1H^b$	$^1H^c$	$^{13}C^a$
1	6.35 (d, 1.5)	6.27 (d, 1.5)	6.24 (d, 1.5)	146.89
2	3.22 (ddd, 4.6, 4.0, 2.2)	3.15 (ddd, 4.8, 4.0, 2.3)	2.60 (m)	41.18
3	2.05 (m)	2.15 (m)	1.70 (m)	63.90
4				212.87
5α	2.54 (dd, 16.6, 4.5)	2.53 (dd, 16.4, 4.4)	2.01 (dd, 16.6, 4.6)	41.04
5β	2.81 (dd, 16.6, 3.4)	2.83 (dd, 16.4, 3.7)	2.53 (dd, 16.6, 3.8)	
6	2.28 (m)	2.17 (m)	1.65 (m)	39.24
7	2.05 (m)	1.94 (m)	1.87 (m)	28.10
8α	2.08 (dd, 8.8, 4.2)	2.14 (dd, 9.2, 4.2)	1.83+ (br d, 9.0)	45.80
8β	2.20 (d, 8.8)	2.19 (d, 9.2)	1.93+ (br d, 9.0)	
9				198.10
10				136.00
11	2.00 (m)	1.98 (m)	1.58 (m)	31.78
12	0.88* (d, 6.1)	0.89* (d, 6.3)	0.75* (d, 6.4)	19.97*
13	1.08* (d, 6.1)	1.03* (d, 6.3)	0.77* (d, 6.4)	20.28*
14	1.02 (d, 6.4)	0.87 (d, 6.5)	0.57 (d, 6.2)	20.93
15	1.73 (d, 1.5)	1.77 (dd, 2.0, 1.4)	1.69 (d, 1.5)	15.45

[a] Recorded in $CDCL_3$. [b] Recorded in pyridine-d_6. [c] Recorded in C_6D_6. *,+ Assignments may be interchanged; proton chemical shifts are reported at δ values (ppm) from internal TMS at 300 MHz. Carbon chemical shifts are reported as δ values (ppm) at 90.8 MHz.

only in the COSY spectrum for the 2-H signal with 3-H at the δ 2.05, indicated the near 90° torsion angle between these two hydrogens. Consequently, the orientation of the methyl group at C-3 was deduced to be the same as that of the hydrogens at the anellation positions (2-H and 6-H). The 8-H_2 methylene protons showed a characteristic coupling pattern; the signal at δ 2.03 was a doublet of doublets, while the geminally coupled signal at δ 2.20 was a doublet, since only one of these two signals shows coupling with 7-H (δ 2.05). The dd pair of 5-H_2 at δ 2.54 and 2.81, however, shows strong geminal coupling, and both signals are coupled with the resonance for 6-H at δ 2.28.

The relative stereochemistry of the substituents as well as certain conformational features of **1** were further established by NOE experiments. Irradiation of the allylic 2-H at δ 3.22 enhanced the C-3 methyl and 6-H methine signals, thereby supporting the cis orientation of these three substituents. No NOE enhancement could be expected between the allylic hydrogen and the methyl group in the case of cadinanes **2** and **3** due to the equatorial position of the methyl group. Irradiation of 1-H at δ 6.35 resulted in area increases at δ 2.05 (3-H) and δ 1.73 (10-CH_3), but no NOE effect was observed between 1-H and 3-CH_3. Separate irradiations of the two dd signals of 5-H_2 established the stereotopical nature of the two nonequivalent hydrogens. In the alternative cadinane structure no NOE interaction could be expected between the isopropyl methyl group and any of the methylene protons, assuming that in the thermodynamically preferred conformation the isopropyl group is equatorial. Irradiation of the signal at δ 2.81 resulted in an area increase for the isopropyl methyl groups, but irradiation of the signal at δ 2.54 did not result in any NOE effects being observed.

Further evidence for the unusual carbon framework and substitution pattern of eupatorenone (1) came from selective INEPT[9] experiments, which also permitted the unambiguous assignment of the ^{13}C NMR spectrum. The APT spectrum of **1** showed four methyl (δ 15.45, 19.97, 20.28, and 20.93), two methylene (δ 41.04 and 42.18), six methine (δ 28.10, 31.78, 39.24, 42.18, 63.90, and 146.89), and three quaternary carbons (δ 136.00, 198.10, and 212.87), of which the latter two could be assigned as carbonyl carbons. Irradiation of 10-CH_3 resulted in enhancements of δ 198.10 and 146.89, which could be assigned as C-9 and C-1, respectively, and irradiation of the isopropyl methyl group enhanced the aliphatic methine (C-7)

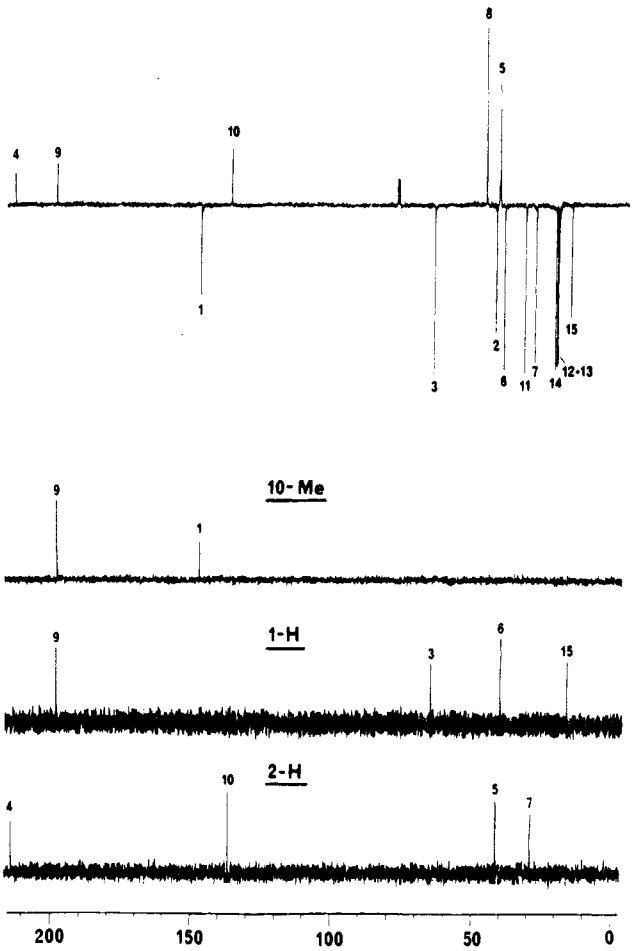

Figure 1. APT and selective INEPT spectra of eupatorenone (1).

at δ 28.10. Magnetization transfer via irradiation of 2-H with ^3J = 6 Hz confirmed the position of the carbonyl function in the five-membered ring leading to enhancements at δ 212.87 (C-4), 136.00 (C-10), 41.04 (C-5), and 28.10 (C-7). Finally, irradiation of 1-H enhanced C-9 at δ 198.10, thereby, confirming the carbonyl placement in the seven-membered ring, C-3 at δ 63.90, C-6 at δ 39.24, and C-15 at δ 15.45 (Figure 1). The assignments for the protonated carbons obtained from the selective INEPT experiments were fully in agreement with the HETCOR spectrum of eupatorenone (1), and the complete assign-

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ments are shown in Table I.

Unlike saturated ketones or isolated olefins, α,β-unsaturated ketones are regarded as a class of inherently dissymmetric chromophores;[10,11] consequently, no sector rules were applicable for the determination of the absolute configuration of the chiral centers of eupatorenone. The signs of the n–π^* (R band, 320–350 nm) and π–π^* (K band, 220–260 nm) transitions of *trans*-enones, however, have been correlated with the sense of helicity for this dissymmetric type chromophore. Thus, the R band is positive and the K band is negative in the case where the helicity of the chromophore is skewed in a left-handed helix; they are opposite when the chromophore helicity is right-handed.[12,13] Since eupatorenone (1) exhibited a CD spectrum characteristic for the presence of a right-handed helicity of the *trans*-enone chromophore, the absolute configuration of the attached C-2 chirality center was established as *S*. Experimentally, the CD values in methanol were $[\theta]$ +2150 and −840 at 248 and 300 nm, respectively. On this basis, and according to the prior determination of the relative steric positions of 2-H, 6-H, 3-CH_3, and 7-CH_3, the absolute configurations of the stereo centers of 1 are proposed as 2*S*, 3*S*, 6*S*, and 7*R*.

It should be noted that the reported ^{1}H NMR data of one of the cadinanes isolated previously[6] closely resembled those of eupatorenone (1). Unfortunately, no ^{1}H–^{1}H COSY measurements or ^{13}C NMR data are available for this compound, which might establish the relationship between this sesquiterpene and eupatorenone (1) or the possible identity of the two compounds.

Eupatorenone (1) was evaluated for cytotoxicity in the KB and P-388 test systems according to established protocols,[14,15] but was inactive.

Experimental Section

Melting point was determined on a Kofler-type hot-stage apparatus and is uncorrected. Optical rotation was measured with a Perkin-Elmer 241 polarimeter. Ultraviolet spectra were recorded with a Beckman DU-7 spectrophotometer, and infrared spectra were obtained with a Nicolet MX-1 interferometer. Mass spectrum was determined on a Varian MAT 112S double-focusing mass spectrometer at 80 eV. The ^{1}H NMR spectra were obtained with either a Nicolet NMC 360 instrument operating at 360 MHz or a Varian XL-300 instrument operating at 300 MHz. The ^{13}C NMR measurements were recorded with the Nicolet NMC 360 instrument operating at 90.8 MHz. Tetramethylsilane (TMS) was used as the internal standard and chemical shifts are reported as δ values (ppm). Homonuclear COSY spectra and heteronuclear HETCOR spectra were recorded with the Varian XL-300 spectrometer. Standard Varian pulse sequences were used. The selective INEPT experiments were performed on the Nicolet NMC 360 spectrometer. Data sets of 16K covering a spectral width of 10 000 Hz were acquired. Proton pulse widths were calibrated by using a sample of acetic acid in 10% C_6D_6 (^1J = 6.7 Hz) in a 5-mm NMR tube.[16] The radio frequency field strength for the soft proton pulse was on the order of 25 Hz in these experiments. For 1-H and 2-H protons, 6 Hz was used as the ^3J value and 4 Hz was used for the irradiation of the allylic and the isopropyl methyl group protons. Five thousand acquisitions were accumulated in each irradiation.

Plant Material. *Eupatorium adenophorum* Spreng. (*Ageratina adenophora* (Spreng.) R. King and H. Robinson) was collected from Doi Suthep, Chiang Mai Province, Thailand, in May 1987. Authentication was performed by comparison with herbarium specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand. A voucher specimen of the plant material was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Isolation of Eupatorenone (1). The powdered dried plant material (2.3 kg) was extracted with 95% EtOH (20 L), and the combined extracts were evaporated in vacuo. The residue was distributed between H_2O (5 L) and petrol (3 × 2 L), and the organic layer was dried and evaporated to a residue (40 g), which was chromatographed on Si gel column, eluting first with benzene and later with benzene containing increasing amounts of acetone to 2, 4, 5, 10, and 15%, respectively. The fractions were evaporated, examined by TLC, and purified further through prep TLC to yield eupatorenone (1) (120 mg, 0.003%) having the following physical and spectroscopic properties: mp 66–67 °C; $[\alpha]_D$ +72.2° (MeOH, *c* 1.3); UV (MeOH) λ_{max} (log ϵ) 242 (3.86) nm; IR (KBr) ν_{max} 1725, 1710, and 1605 cm^{-1}; ^{1}H NMR, see Table I; ^{13}C NMR, see Table I; mass spectrum, *m/z* (rel intensity) 234 (M^+, 22), 232 (6), 216 (10), 192 (41), 117 (6), 164 (9), 150 (45), 136 (84), 121 (28), 109 (24), 69 (100), 55 (26); CD (MeOH) $[\theta]_{248}$ +2150; $[\theta]_{300}$ −840.

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Studies on the Extent of Electron Delocalization in β-Nitro Enamines from Dipole Moment Measurements

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Full delocalization of π-electrons in a tertiary enamine system such as described by structure 1 requires the nitrogen lone-pair orbital and the alkene π-system orbitals to be parallel in order to maximize overlap.[1] However,

$$R_2N{-}CH{=}CH_2 \leftrightarrow R_2\overset{+}{N}{=}CH{-}CH_2^-$$
1

most tertiary enamines do not possess such a completely conjugated system. Rather, the enamine is usually distorted from this planar geometry due to some pyramidality of the enamine nitrogen and some torsional twist away from planarity around the carbon–nitrogen bond.[2]

A viable method for determining the extent of π-electron delocalization in a conjugated system is comparing its experimental dipole moment with the dipole moment of a similar compound where mesomerism is excluded or with

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