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KOPSOFFINE: A NEW DIMERIC INDOLE ALKALOID OF PLEIOMUTINE TYPE FROM *KOPSIA OFFICINALIS*

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ABSTRACT.—A new dimeric indole alkaloid of the 1-norpleiomutine type, (+)-kopsoffine, has been isolated from the roots of *Kopsia officinalis*. The proposed structure was confirmed by partial synthesis. The dihydroeburnamenine moiety of (+)-kopsoffine was found to be the optical antipode of that present in (−)-pleiomutine. Detailed ^{1}H-nmr data of (−)-isoeburnamine, (+)-eburnamine, and (−)-eburnamonine are also presented.

The plants of the genus *Kopsia* (Apocynaceae) are represented in China, India, Thailand, Malaysia, Indonesia, and the Philippines. Four species (two cultivated) are known to be present in China (1). One of them, *Kopsia officinalis* Tsiang et P.T. Li, is a tall evergreen tree, endemic to the Yunan province in southern China. The plant has been used in Chinese folk medicine for the treatment of dropsy, rheumatoid arthritis, and as an analgesic in pharyngitis and tonsillitis (2). The results of preliminary pharmacological experiments in the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China, have shown that the total alkaloids of *K. officinalis* exhibit analgesic action in mice without sedative effects after oral administration (3).

RESULTS AND DISCUSSION

The total alkaloids obtained by alcoholic extraction of the powdered roots of *K. officinalis* (4) were fractionated into monomers and dimers by Sephadex LH-20 column chromatography. From the dimeric part, we isolated a new dimeric indole alkaloid, with the structure **1a**, for which we propose the name (+)-kopsoffine.

(+)-Kopsoffine (**1a**), m.p. 240°, $[\alpha]_D$+4° (c=1.1, $CHCl_3$), cd $\Delta\epsilon_{300}$+2.02, $\Delta\epsilon_{330}$−0.65 (c=0.2, EtOH), ir ($CHCl_3$) 3380 (NH), 1730 (C=O), 1610 (C=C) cm^{-1}, uv λ max (EtOH) 234 (4.44), 259 (3.96), 287 (3.84), 292 (3.84) nm (indolic and dihydroindolic chromophores).

The mass spectrum showed a molecular ion at *m/e* 616 corresponding to $C_{40}H_{48}N_4O_2$ (high resolution analysis: calcd 616.3777; found: 616.3760). Intense peaks at *m/e* 124 and 109 suggested the presence of a pleiocarpine-type moiety in the dimer (5). In general, the fragmentation was analogous to that of (−)-pleiomutine (**2b**)[1] (6,7), whose molecular ion is 14 units higher than that of kopsoffine (**1a**). As the ^{1}H-nmr spectrum (see below) indicated the absence of any >N-CH_3 group (no 3H singlet at *ca.* δ 2.8), we took as a working hypothesis that (+)-kopsoffine might be identical to one of the isomers of 1-nor-pleiomutine[1] [where a dihydroeburnamenine

[1]For a revised stereostructure of (−)-pleiomutine (**2b**), see reference (9).

moiety is linked with a kopsinine (1-nor-pleiocarpinine) moiety].[2] Indeed, the methylation of (+)-kopsoffine afforded a compound **1b**, which showed the same fragments in the mass spectrum as (−)-pleiomutine (**2b**). The difference in $[\alpha]_D$ values between (+)-kopsoffine (**1a**) (+4°—see above), (−)-nor-pleiomutine (**2a**) (−65°) (8) and (−)-pleiomutine (**2b**) (−97°) (6) (−111°) (7), strongly suggested a difference in the stereochemistry between (+)-kopsoffine (**1a**) and (−)-pleiomutines **2a** and **2b**.

The ^{1}H-nmr spectrum (Table 1) was in full agreement with the suggested structure. The protons of the ethyl side chain of the dihydroeburnamenine moiety produced a three-proton triplet resonance at δ 0.87 and two one-proton doublets of quartets resonances at δ 1.47 and δ 2.15. The proton at C-16′ gave rise to a signal at δ 4.98 (1H, dd, J=11 Hz, J=5 Hz). The observed splittings are consistent with ax-ax and ax-eq couplings and indicate that C-16′H is axial (7). The C-10 linkage position in the kopsinine moiety was determined by analysis of the aromatic region of the spectrum (Table 1) (10). The presence of a methoxycarbonyl group was indicated by the three-proton singlet resonance at δ 3.78.

Further proof of the correctness of structure **1a** for (+)-kopsoffine was obtained by ^{13}C-nmr spectroscopy (Table 2). Monomeric indole alkaloids (−)-kopsinine (**4**) and (+)-vincamine (**5**) were used as reference compounds.

The proposed stereostructure **1a** was confirmed finally by partial synthesis according to Büchi *et al.* (13). Condensation of a (+)-eburnamine (**6**)/(−)-isoeburnamine (**3**) (a trace) mixture,[3] prepared from (−)-eburnamonine (**7**) by $LiAlH_4$ reduction (see below), and (−)-kopsinine (**4**) (14) under acidic conditions, led to a product that proved to be identical to the natural product (tlc, $[\alpha]_D$, cd, uv, ir, ^{1}H-nmr, ms). In

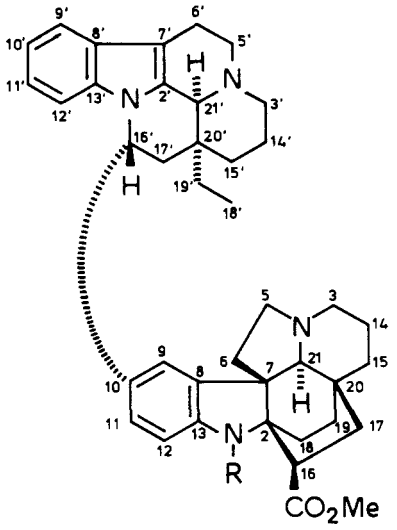
TABLE 1. ^{1}H-nmr Data of Kopsoffine (**1a**)[a]

H-9	7.18 s
H-11	6.89 br d
H-12	6.65 d
H-21	3.00 s
CO_2Me	3.78 s
H-9′	7.46 d
H-10′	7.01 t
H-11′	6.84 t
H-12′	6.55 d
H-16′	4.98 dd
H-17′	2.11 dd
H-17′	1.79 dd
H-18′ (Me)	0.87 t
H-19′	1.47 dq
H-19′	2.15 dq
H-21′	4.03 s

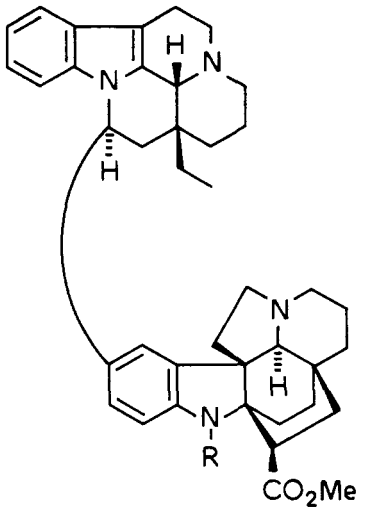
[a]The spectrum was run in $CDCl_3$. Values are in ppm downfield from TMS, s, singlet; d, doublet; t, triplet; q, quartet; br, broad.

[2]The presence of (−)-isoeburnamine (**3**) and (−)-kopsinine (**4**) in the monomeric fraction of the total alkaloids from *K. officinalis* (14), strongly supports this hypothesis. On the other hand, the presence of **3** and **4** might raise the question whether (+)-kopsoffine (**1a**) could be an artifact. However, the mild procedure used in the isolation of **1a** (no acidic conditions) makes this alternative unlikely.

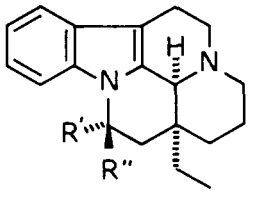
[3]Both (+)-eburnamine (**6**) and (−)-isoburnamine (**3**) give under the used reaction conditions the same reaction intermediate **8**.



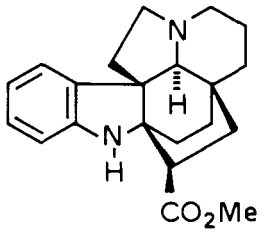
1a R=H
1b R=CH_3



2a R=H
2b R=CH_3



3 R′=H, R″=OH
5 R′=CO_2Me, R″=OH
6 R′=OH, R″=H
7 R′, R″ =O



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particular, the coincidence of the $[\alpha]_D$ values and the superposition of the circular dichroism curves (see above) were important for confirmation of the stereochemistry suggested for (+)-kopsoffine (**1a**). Thus, the dihydroeburnamenine moiety of (+)-kopsoffine (**1a**) is the optical antipode of that present in (−)-pleiomutines **2a** and **2b**.

To obtain useful ^{1}H-nmr data for structural determinations of further indole alkaloids of the eburnamine type, a detailed ^{1}H-nmr study (400 MHz) of (−)-isoeburnamine (**3**). (+)-eburnamine (**6**), and (−)-eburnamonine (**7**) (see above) was also undertaken. Application of consecutive double resonance experiments and comparison with the earlier ^{1}H-nmr data allowed all of the protons in compounds **3, 6,** and **7** to be assigned (Table 3).

EXPERIMENTAL

The ir spectra were recorded on a Perkin-Elmer 257 spectrophotometer, uv spectra on a Bausch and Lomb spectrophotometer, cd spectra on a Roussel-Jouan dichrograph, and ^{1}H-nmr spectra on the I.E.F. 400 (400 MHz) instrument of the Institut d'Electronique Fondamentale d'Orsay (15). Mass spectra were recorded on an A.E.I. MS-50 mass spectrometer at 70 eV using direct sample insertion into the ion source, whose temperature was 160-180°.

TABLE 2. The ^{13}C-nmr Data of Kopsoffine (**1a**), Kopsinine (**4**), and Vincamine (**5**)

	kopsoffine	kopsinine[a]		kopsoffine	vincamine[b]
C-2	67.1	66.7	C-2′	134.8	131.4
C-3	47.7	47.6	C-3′	44.7	44.5
C-5	51.1	50.7	C-5′	51.1	50.9
C-6	36.6	36.5	C-6′	16.8	16.9
C-7	58.4	57.9	C-7′	104.9	105.9
C-8	136.9	140.6	C-8′	128.8	128.9
C-9	120.4	121.6	C-9′	117.8	118.4
C-10	141.8	119.7	C-10′	120.4	121.5
C-11	125.8	126.6	C-11′	119.2	120.1
C-12	112.5	110.8	C-12′	111.3	110.2
C-13	148.9	149.0	C-13′	134.3	134.1
C-14	17.2	17.1	C-14′	20.9	20.8
C-15	34.9[c]	34.8[d]	C-15′	24.5	25.2
C-16	43.9	43.8	C-16′	56.2	81.9
C-17	31.8[c]	31.8[d]	C-17′	45.3	44.5
C-18	34.0[c]	33.9[d]	C-18′	7.6	7.6
C-19	34.0[c]	33.9[d]	C-19′	28.9	28.8
C-20	32.4	31.2	C-20′	35.1	35.1
C-21	68.2	68.4	C-21′	59.8	59.1
C=O	175.1	174.3	C=O		174.3
OMe	52.1	51.9	OMe		54.1

The spectra were recorded in $CDCl_3$ solution. The δ values are in ppm downfield from TMS.
[a]The interpretation of the signals is mainly based on the results given for venalstonine (11).
[b]Taken from reference (12).
[c,d]Signals may be interchanged.

TABLE 3. ^{1}H-nmr Data of (−)-Isoeburnamine (**3**), (+)-Eburnamine (**6**), and (−)-Eburnamonine (**7**)[a]

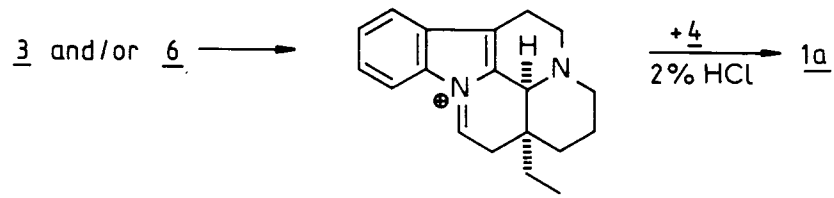
	3	**6**	**7**
H_R-3[b]	2.61 br d	2.46 br d	2.58 br d
H_S-3	2.67 br d	2.28 br dd	2.35 br dd
H_R-5	3.36 dd	3.20 dd	3.28 dd
H_S-5	3.26 ddd	3.28 ddd	3.16 ddd
H_R-6	3.00 m	2.90 m	2.86 m
H_S-6	2.55 br d	2.48 br d	2.41 br d
H-9	7.41 d	7.46 d	7.40 d
H-10	7.15 t	7.14 t	7.24 t
H-11	7.19 t	7.17 t	7.28 t
H-12	7.51 d	7.73 d	8.35 d
H_R-14	1.77 br ddd	1.66 br ddd	1.72 br ddd
H_S14	1.40 br d	1.27 br d	1.38 br d
H_R-15	1.64 ddd	0.79 ddd	0.98 ddd
H_S-15	1.55 br d	1.33 br d	1.47 br d
H-16	6.07 d	5.51 dd	—
H_R-17	2.00 dd	2.21 dd	2.55 def. d
H_S-17	2.19 d	1.42 dd	2.62 def. d
H-18	0.93 t	0.87 t	0.94 t
H-19	1.47 d q	1.37 d q	1.62 d q
H-19′	2.17 d q	2.00 d q	2.02 d q
H-21	3.85 s	3.50 s	3.85 s

[a]The spectra were run in $CDCl_3$ at 400 MHz. Values are in ppm downfield from TMS: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; def., deformed.
[b]The Hanson prochirality nomenclature system (16,17) is applied to distinguish between the H-atoms of different -CH_2- groups.

EXTRACTION AND PURIFICATION.—The plant material used was collected in May 1979 in Yunan province, China. The botanical identification of this material as *Kopsia officinalis* was made at the Department of Medicinal Plants (Botany) of the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Alcoholic extraction of the air-dried root powder of *K. officinalis* in the classical manner gave the total alkaloids in a 0.34% yield. The monomeric and dimeric alkaloids were separated by filtration on Sephadex LH-20 column. Further purification of the dimer was effected by column chromatography (alumina), followed by preparative layer chromatography (silica gel) and recrystallization from hexane-Me_2CO (1.07% of total alkaloids).

PARTIAL SYNTHESIS OF (+)-KOPSOFFINE (**1a**).—The reaction of $LiAlH_4$ with (−)-eburnamonine (**7**) (250 mg) in dry THF led to a mixture (210 mg) of (+)-eburnamine (**6**) and (−)-isoeburnamine (**3**) (trace amounts). This mixture (200 mg) and (−)-kopsinine (**4**) (200 mg) in a 2% HCl/MeOH solution was refluxed for 4 h. The cooled reaction mixture was neutralized with Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ solution was dried over Na_2SO_4 and evaporated under vacuum to dryness. The residue was purified by PLC (silica gel) to yield 96 mg (26%) of a product which proved to be identical with the natural product **1a**.



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Scheme 1.

METHYLATION OF (+)-KOPSOFFINE (**1a**).—Kopsoffine (**1a**) (100 mg) was suspended in an aqueous solution of formaldehyde at 0° buffered at pH 4; $NaBH_4$ (200 mg) was added in small portions to this suspension, and it was stirred for 6 h. The reaction was then stopped by dilution with aqueous Na_2CO_3 solution and extracted with CH_2Cl_2.

The combined organic fractions were washed with H_2O, dried over anhydrous Na_2SO_4, and distilled. On purification of the crude product on preparative tlc, pure *N*-methyl-kopsoffine (**1b**) was obtained as an amorphous product: $[\alpha]^{20}D-7°$ (C=1.8, $CHCl_3$); ms (relative intensity) *m/e*: 630 ($M^{+\cdot}$, 100), 252 (40), 250 (25).

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