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17-α-Hydroxy-$\Delta^{14,15}$-kopsinine and a bisindole alkaloid from *Kopsia teoi*

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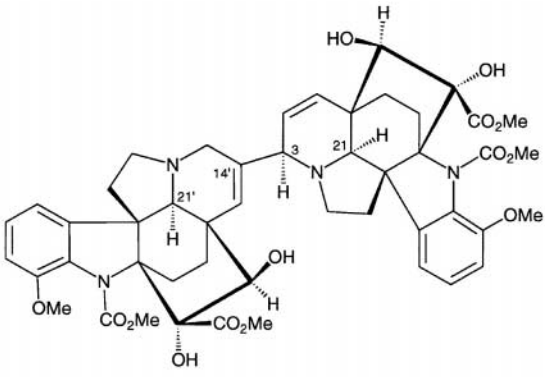
Abstract

The structure of 17-α-hydroxy-$\Delta^{14,15}$-kopsinine has been confirmed by a detailed NMR analysis. A bisindole alkaloid was obtained from the leaf extract of *Kopsia teoi* and its structure established by spectral methods. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Kopsia teoi*; Indole alkaloids

1. Introduction

We first reported the isolation and structure determination of 17-α-hydroxy-$\Delta^{14,15}$-kopsinine **1**, which was obtained from the stem-bark extract of *Kopsia teoi* L. Allorge, a Malayan *Kopsia* (Kam, Yoganathan, Chuah, & Chen, 1993). This species has also provided a variety of new indole alkaloids, especially those possessing an aspidofractinine skeleton (Kam, Yoganathan, & Chuah, 1993; Kam, Yoganathan, & Chuah, 1994; Kam, Yoganathan, & Chen, 1996; Kam & Yoganathan, 1996; Kam, Yoganathan, & Mok, 1997). The NMR spectral data indicated that **1** is an aspidofractinine-type compound with an unsubstituted aromatic ring, the presence of C(16) methyl ester, C(17)-hydroxyl functions, unsaturation at C(14) and C(15) and the absence of carbamate and C(16)–OH groups. The stereochemistry of the C(17)–OH was deduced to be α from the observed W-coupling (2 Hz) between H(17β) and H(19α), while the absence of similar coupling between H(16) and one of the H(18) indicated that the C(16) methyl ester group was β, which was also in accord with the observed H(16)/H(17) coupling of 7.5 Hz (Kam et al., 1993). In another study of the same species (Varea et al., 1993), a com-



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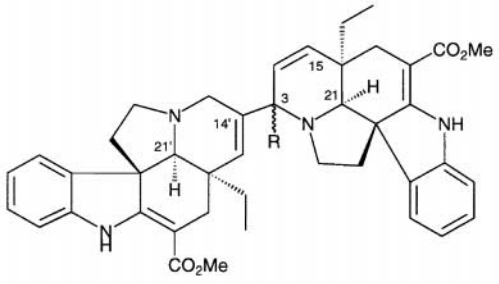

4 R = α-H
5 R = β-H

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pound constituted as shown in **2** (16-*epi*-17α-hydroxy-$\Delta^{14,15}$-kopsinine) with virtually identical spectral data was reported, but in which W-coupling between H(16) and H(18) was apparently detected, requiring the methyl ester function to be α. In the light of these uncertainties (Saxton, 1995), we were prompted to undertake a reinvestigation of this compound, since it is clear from examination of the spectral and other data, that these two compounds which were obtained from the same plant, are identical, and the two structures put forward differed only with respect to the stereochemistry at C(16). We now report our findings, which have vindicated the original assignment of this compound as 17α-hydroxy-$\Delta^{14,15}$-kopsinine **1**. We also report full details for the isolation and structure elucidation of the new bisindole alkaloid, nitaphylline **3** (Kam & Yoganathan, 1997).

2. Results and discussion

The ^{1}H NMR spectrum of **1** was obtained again at 400 MHz (which resolved most of the peaks in contrast to the earlier spectra obtained at 300 MHz, see Table 1) and detailed analysis was carried out by application of COSY, homonuclear decoupling and NOE. In the earlier two studies (Kam et al., 1993; Varea et al., 1993), the stereochemistry of the hydrogens at C(18) and C(19) were not assigned, which is now necessary, since W-coupling, if it occurs as reported for H(16), can only take place between H(16β) and H(18α). There are two signals which are attributed to H(18), viz., δ 1.30 and 1.88, which have been previously established by COSY and HMQC. Similarly, the H(19) signals are at δ 1.12 (19α) and 2.11 (19β). The W-coupling observed between H(17β) and H(19α) was common in both the two previous reports (Kam et al., 1993; Varea et al., 1993) and this allowed assignment of the H(19) signals as shown. Irradiation of H(19α) resulted in NOE enhancement of H(21) and the H(18) signal at δ 1.88 and vice versa. This allowed assignment of H(18α) to the signal at δ 1.88 and H(18β) to the signal at δ 1.30, which are also in agreement with the other NOE's observed (Table 1). In the paper by the French investigators (Varea et al., 1993), it was reported that the H(18) signal at δ 1.30, which we now determine to be β, showed W-coupling to H(16β), which clearly cannot be the case since W-coupling, if it occurs at all, can only take place between H(16β) and H(18α). Be that as it may, the key experimental result that provides incontrovertible evidence for H(16α) stereochemistry is the observed NOE enhancement of H(16α) on irradiation of H(18β) and vice versa (Fig. 1). This interaction, even if the H(18) and H(16) signals were not assigned any stereochemistry, is only possible between H(18β) and H(16α). Had the H(16) been β, NOE would have been impossible with either of the C(18) hydrogens. In addition, the

Table 1
^{1}H NMR spectral data for compound **1** ($CDCl_3$)

H	δ_H (Kam et al., 1993)[a]	δ_H (Varea et al., 1993)[b]	δ_H (400 MHz)	NOE[c]
3	3.38–3.56 m	3.43 m	3.43 dt (J_{3-3} = 16.7, J_{3-14} = 2.1, J_{3-15} = 2.1)	
3	3.38–3.56 m	3.43 m	3.51 ddd (J_{3-3} = 16.7, J_{3-14} = 3.7, J_{3-15} = 2.1)	
5α	2.72–2.86 m	2.70–2.80 m	2.80 ddd ($J_{5\alpha-6\beta}$ = 9.8, $J_{5\alpha-5\beta}$ = 8.2, $J_{5\alpha-6\alpha}$ = 4.7)	6α
5β	2.96 brt (7)	2.96 ddd (8.2, 6.6, 1.5)	2.97 ddd ($J_{5\beta-5\alpha}$ = 8.2, $J_{5\beta-6\beta}$ = 6.5, $J_{5\beta-6\alpha}$ = 1.5)	6β, 17β
6α	1.21–1.36 m	1.31 m	1.30 ddd ($J_{6\alpha-6\beta}$ = 12, $J_{6\alpha-5\alpha}$ = 4.7, $J_{6\alpha-5\beta}$ = 1.5)	5α
6β	2.47 ddd (12, 10, 7)	2.45 ddd (12.2, 9.8, 6.6)	2.47 ddd ($J_{6\beta-6\alpha}$ = 12, $J_{6\beta-5\alpha}$ = 9.8, $J_{6\beta-5\beta}$ = 6.5)	5β, 17β
9	7.04 d (7)	6.7–7.0 m	7.05 d (7.5)	
10	6.74 t (7)	6.7–7.0 m	6.74 td (7.5, 1)	
11	7.01 t (7)	6.7–7.0 m	7.03 td (7.5, 1)	
12	6.71 d (7)	6.7–7.0 m	6.70 d (7.5)	
14	5.89 dt (10, 3)	5.87 ddd (9.9, 3.3, 2.5)	5.89 ddd (J_{14-15} = 10, J_{14-3} = 3.7, J_{14-3} = 2.1)	
15	5.66 dt (10, 2)	5.64 dt (9.9, 2.1)	5.66 dt (J_{15-14} = 10, J_{15-3} = 2.1, J_{15-3} = 2.1)	
16α	2.77 d (7.5)	2.77 dd (7.7, 2,1)	2.77 dd ($J_{16\alpha-17\beta}$ = 7.7, $J_{16\alpha-18\alpha}$ = 1.1)	18β
17β	5.01 dd (7.5, 2)	5.00 dd (7.7, 2)	5.02 dd ($J_{17\beta-16\alpha}$ = 7.7, $J_{17\beta-19\alpha}$ = 2.1)	6β
18α	1.88 ddd (12, 11.5, 7.5)	1.87 ddd (12.8, 11, 7.5)	1.88 dddd ($J_{18\alpha-18\beta}$ = 13, $J_{18\alpha-19\alpha}$ = 11, $J_{18\alpha-19\beta}$ = 7.5, $J_{18\alpha-16\alpha}$ = 1.1)	19α, 21
18β	1.21–1.36 m	1.30 ddt (12.8, 10.9, 2.1)	1.30 ddd ($J_{18\beta-18\alpha}$ = 13, $J_{18\beta-19\beta}$ = 11, $J_{18\beta-19\alpha}$ = 2.1)	16α, 19β
19α	1.12 brt (12)	1.11 ddt (12.7, 11, 2.1)	1.12 ddt ($J_{19\alpha-19\beta}$ = 13, $J_{19\alpha-18\alpha}$ = 11, $J_{19\alpha-18\beta}$ = 2.1, $J_{19\alpha-17\beta}$ = 2.1)	15, 18α, 21
19β	2.09 ddd (12, 11.5, 7.5)	2.10 ddd (12.7, 10.9, 7.4)	2.11 ddd ($J_{19\beta-19\alpha}$ = 13, $J_{19\beta-18\beta}$ = 11, $J_{19\beta-18\alpha}$ = 7.5)	16α, 18β
21	2.72 s	2.73 s	2.72 s	9, 19α
OMe	3.78 s	3.77 s	3.79 s	

[a]$CDCl_3$, 270 MHz.
[b]$CDCl_3$, 300 MHz.
[c]NOE's of geminal hydrogens not indicated.

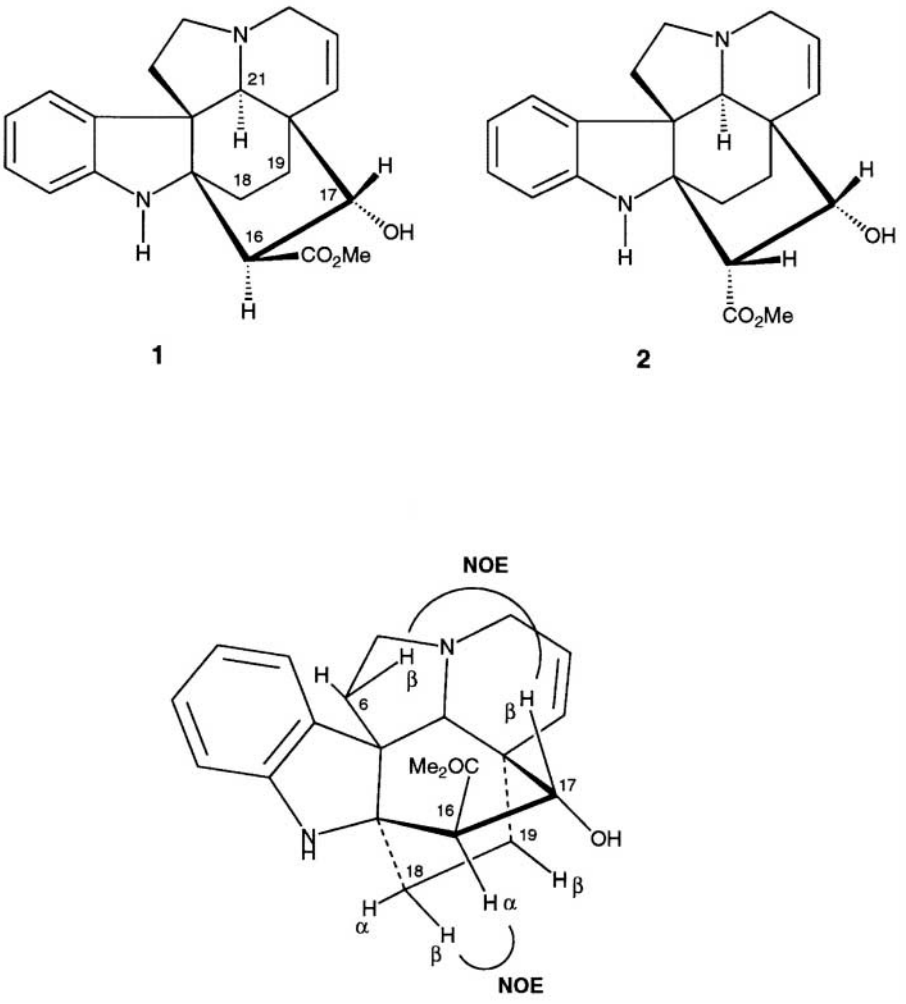

Fig. 1.

400 MHz ^{1}H NMR spectrum fully resolved the H(18) and H(16α) signals as follows: δ 1.30, H(18β), ddd ($J_{18–18}$ 13 Hz, $J_{18β–19β}$ 11 Hz, $J_{18β–19α}$ 2.1 Hz); δ 1.88, H(18α), dddd ($J_{18–18}$ 13 Hz, $J_{18α–19α}$ 11 Hz, $J_{18α–19β}$ 7.5 Hz, $^4J_{18α–16}$ 1.1 Hz); δ 2.77, H(16α), dd ($J_{16–17}$ 7.7 Hz and $^4J_{16–18α}$ 1.1 Hz). The small 1.1 Hz coupling observed for H(18α) and H(16α) is due to long-range coupling ($^4J_{16–18α}$), which was readily confirmed by homonuclear decoupling. Thus, irradiation of the H(16α) signal at δ 2.77 resulted in the collapse of the H(18α) signal at δ 1.88 (dddd) to a ddd with J 13, 11, 7.5 Hz and, similarly, irradiation of the H(18α) signal resulted in the collapse of the H(16α) doublet of doublets to a doublet (J 7.7 Hz). The present results have therefore completely vindicated the original assignment of this compound as 17-α-hydroxy-$\Delta^{14,15}$-kopsinine **1** (Kam et al., 1993).

Compound **3**, nitaphylline was obtained from the leaf extract of *Kopsia teoi* and was not present in the stem-bark. The EI mass spectrum indicated a bisindole alkaloid ([M$^+$] m/z 910, base) and high resolution measurements gave the formula $C_{48}H_{54}N_4O_{14}$ (see Section 3). The UV spectrum of **3** was virtually superimposable on that of kopsingine, providing early indication that it could be constituted from the union of two kopsingine units. This was also indicated by the mass spectrum which showed a significant peak at m/z 455 which can be ascribed to cleavage of the parent ion along the bond connecting the monomeric moieties. This supposition was confirmed on examination of the ^{1}H and ^{13}C NMR spectra. The ^{13}C NMR spectrum of **3** (Table 2) accounted for a total of only 33 peaks, indicating overlap of 15 carbon resonances. Among the distinguishable signals, four pairs are only just so, differing in chemical shift by ca. 0.1 or 0.2 ppm. The bulk of the overlapped signals are readily assigned to the aromatic portions of the alkaloid as well as the methoxyl, carbamate and ester groups and are similar to those of kopsingine (Kam et al., 1993). Similarly, based on comparison with kopsingine, as well as standard 2D experiments, the remaining resonances can be assigned to carbons 2, 5, 6, 7, 16, 17, 18,

Table 2
^{1}H and ^{13}C NMR spectral data for compound **3**[a]

Position	δ_C	δ_H	Position	δ_C	δ_H
2	76.1[b]	–	2′	76.0[b]	–
3	66.1	ca. 3.78	3′	48.6	3.11 d (16)
		–			3.32 d (16)
5	46.7[c]	2.56 m[i]	5′	48.6[c]	2.58 m[i]
		2.93 m			2.93 m
6	40.6[d]	1.73 dd (13, 4)	6′	40.0[d]	1.73 dd (13, 4)
		3.11 m[j]			3.09 m[j]
7	56.2[e]	–	7′	56.4[e]	–
8	143.8	–	8′	143.8	–
9	111.8	6.82 br d (7)	9′	111.8	6.82 br d (7)
10	125.0	7.03 br t (7)[k]	10′	125.0	7.04 br t (7)[k]
11	113.2	6.74 br d (7)[l]	11′	113.2	6.77 br d (7)[l]
12	149.2	–	12′	149.2	–
13	128.2	–	13′	128.2	–
14	131.5	5.77 m	14′	139.5	–
15	131.9	5.77 m	15′	133.0	5.77 m
16	79.8	–	16′	79.8	–
17	80.7[f]	ca. 3.77	17′	81.5[f]	ca. 3.77
18	27.0	1.40 m	18′	27.0	1.40 m
		2.10 br t (13)			2.10 br t (13)
19	25.6	1.15 m	19′	25.6	1.15 m
	–	1.71 m		–	1.71 m
20	38.7[g]	–	20′	38.9[g]	–
21	68.6	2.73 d (2)[m]	21′	68.6	2.84 d (2)[m]
ArOMe	55.9	3.82 s	ArOMe′	55.9	3.82 s
CO_2Me	51.9	3.75 s	CO_2Me'	51.9	3.75 s
CO_2Me	171.5[h]	–	CO_2Me'	171.6[h]	–
NCO_2Me	52.9	3.78 s	NCO_2Me'	52.9	3.78 s
NCO_2Me	155.5	–	NCO_2Me'	155.5	–
16-OH	–	5.81 m	16-OH′	–	5.81 m
17-OH	–	7.98 d (6)[n]	17-OH′	–	8.00 d (6)[n]

[a]$CDCl_3$, 270 MHz; assignments based on COSY, HMQC and HMBC.
[b–n]Assignments may be reversed.

19 and 21 (as well as the corresponding carbons 2′, 5′, 6′, 7′, 16′, 17′, 18′, 19′ and 21′) of the basic aspidofractinine framework. This leaves the resonances of the ring D carbons which showed greater departure from kopsingine and form the bulk of the non-overlapping resonances of nitaphylline. This observation suggests that the point of branching of the monomers involves the ring D (piperidine) carbons in both units, which was confirmed by examination of the ^{1}H NMR spectrum. The ^{1}H NMR spectrum (Table 2) shows six aromatic hydrogens (δ 6.74–7.04), three olefinic hydrogens (overlapping multiplets at δ 5.7–5.8), two geminal hydrogens due to an aminomethylene group (δ 3.11 and 3.32), which are well resolved, and only one H due to an aminomethine (δ ca. 3.78). This indicates position 3 of one kopsingine unit and position 14′ or 15′ of the other as candidates for points of connection of the monomeric moieties. The geminal hydrogens on C(3′) are obtained as doublets (J = 16 Hz) with no evidence of coupling to any adjacent olefinic hydrogens (cf. J_{3-14} ca. 4.5 Hz in kopsingine, Kam et al., 1993), indicating C(14′) as the point of branching in the other kopsingine unit. The dimer is therefore constituted from two kopsingine units bridged from C(3) of one to C(14′) of the other. It remains only to determine the stereochemistry at the point of branching, i.e. C(3). Unfortunately, the H(3) aminomethine resonance is buried in the massive methoxyl peak at δ 3.78 and the olefinic proton resonances are not well resolved. Fortunately however, a valuable precedent exists in the literature which allows the stereochemistry at this center to be determined (Stöckigt, Pawelka, Tanahashi, Danieli, & Hull, 1983). In the related bistabersonine dimers, voafrine A **4** and voafrine B **5**, which possess the same mode of connection of the monomeric moieties, it was observed that in voafrine A **4**, where the attachment of the second tabersonine unit is β, the pseudoequatorially oriented substituent is directed away from the other tabersonine unit resulting in reduced spatial interaction between the two halves, whereas in voafrine B **5**, in which the substituent is α (pseudoaxial), there is greater spatial proximity

between the two units (Stöckigt et al., 1983). This difference is reflected in both the ^{1}H and ^{13}C NMR spectral data. In the case where the substituent is β, the chemical shifts of corresponding hydrogens in the two units are very similar, whereas in the case where the substituent is α, these hydrogens show significantly different chemical shifts, especially H(5), H(6), H(9), H(18) and H(21). The same is true in the case of the ^{13}C NMR spectra especially with respect to the C(21) resonance. The β-substituted compound should have very similar C(21) resonances which are also close to that of the monomer itself, whereas the α-substituted compound shows very different chemical shifts for the two C(21) resonances. Examination of models in the case of nitaphylline indicates that a similar situation prevails, viz., the β-substituted epimer has the second kopsingine unit pointing away from the first, while the α-substituted compound results in closer spatial proximity of the two halves, just as in the case of the voafrines. Since the behavior of the H and C resonances in nitaphylline parallel that of voafrine A **4**, showing similar chemical shifts for corresponding hydrogens in the two units and similar carbon shifts for C(21), we conclude that the stereochemistry at C-3 is β as shown in structure **3**. Nitaphylline **3** represents the first example of an aspidofractinine-aspidofractinine type bisindole alkaloid.

3. Experimental

3.1. Plant material

Details of collection, deposition of voucher specimens, etc., have been reported earlier (Kam et al., 1993).

3.2. Extraction and isolation

Extraction of alkaloids was carried out in the usual manner which has been described in detail elsewhere (Kam et al., 1993; Kam & Tan, 1990). The isolation and spectral data of compound **1**, $[\alpha]_D$ −66° ($CHCl_3$, *c* 0.086), have also been previously described (Kam et al., 1993). In the present investigation, ^{1}H NMR spectra of compound **1** were recorded in $CDCl_3$ using TMS as int. standard at 400 MHz. Compound **3**, nitaphylline was obtained from the EtOH extract of the leaves. The crude alkaloidal mixt. from the EtOH extract was first chromatographed over silica gel and eluted with $CHCl_3$ with increasing proportions of MeOH, to give 5 major frs. The middle fr. (fr. 3) which comprised a mixt. of several alkaloids, was subjected to repeated fractionation (CC and centrifugal TLC) to give nitaphylline **3** (yield 0.047 g kg^{-1}). Solvent systems used were $CHCl_3$–MeOH and Et_2O–EtOAc (CC) and Et_2O–EtOAc, 20:1 (centrifugal TLC).

3.3. Nitaphylline 3

$[\alpha]_D$ +136° ($CHCl_3$, *c* 1.05). UV (EtOH), λ_{max} (log ε) 217 (4.55), 254 (4.05), 282 (3.51), 289 (3.49). EIMS, *m*/*z* (rel. int.): 910 $[M]^+$ (100), 852 (15), 793 (44), 549 (40), 455 (18), 427 (5), 396 (5), 367 (4), 337 (22) and 107 (8). HREIMS, M^+ found *m*/*z* 910.3609, calcd for $C_{48}H_{54}N_4O_{14}$ 910.3636. ^{1}H and ^{13}C NMR: Table 2.

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