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AN ALKALOID FROM *HAPLOPHYLLUM TUBERCULATUM*

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Key Word Index—*Haplophyllum tuberculatum*; Rutaceae; alkaloid; (+)-tuberine.

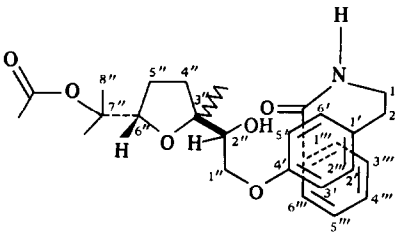
Abstract—A new alkaloid (+)-tuberine was isolated from *Haplophyllum tuberculatum*. Physicochemical and spectral evidence established its structure and stereochemistry as *N*-benzoyl-4′-[(2″*S*,3″*S*,6″*S*)-(+)-7″-acetoxy-2″-hydroxy-3″,7″-dimethyl-3″,6″-epoxyoctyloxy]phenethylamine.

INTRODUCTION

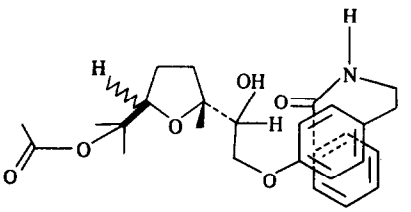
As detailed in our previous paper [1] in which we reported the isolations and structural characterizations of several 1-aryl-2,3-naphthalide lignans, the silica gel chromatography of the chloroform extract from the aerial parts of *Haplophyllum tuberculatum* plants yielded numerous fractions (164). Careful examination by analytical TLC and separation by preparative TLC of the residue from a combination of 50 (85F–135F) of these fractions resulted in the isolation of an alkaloid (**1a**). The constituent, which we named (+)-tuberine, was encountered in nature for the first time and showed strong antibacterial effects on *Staphylococcus aureus* and *Escherichia coli* when examined using the disk diffusion technique in concentrations of 0.1–1.0 μg/ml. The present work is concerned with the elucidation of its structure and stereochemistry.

RESULTS AND DISCUSSION

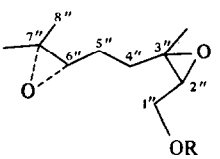
(+)-Tuberine is *N*-benzoyl-4′-[(2″*S*,3″*S*,6″*S*)-(+)-7″-acetoxy-2″-hydroxy-3″,7″-dimethyl-3″,6″-epoxyoctyloxy]-phenethylamine (**1a**). Its structure and stereochemistry were determined by spectral and physicochemical (IR, UV, ^{1}H NMR, ^{13}C NMR, mass spectrometry, $[\alpha]_D$) properties. The IR spectrum showed absorption bands in the regions of hydroxyl (3540 cm^{-1}), secondary amide (3440, 1650 cm^{-1}) and acetoxyl (1720, 1250 cm^{-1}) functions. The UV spectrum indicated the presence of a *N*-benzoyl tyramine chromophore [2, 3]. The alkaloid possessed $[\alpha]_D^{25}$ + 12.5° and *S* absolute stereochemistry at the chiral centre bearing the secondary alcohol function (C-2″). The latter was established by applying Horeau's method [4, 5]. Based on the forthcoming spectral (^{1}H NMR, ^{13}C NMR) evidence, the relative configurations of the substituents



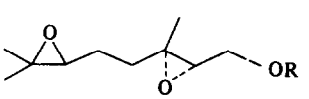
1a



1b



2a



2b

on the tetrahydrofuran ring (C-3″ and C-6″) were assigned *trans* (3″*S*,6″*S* or 3″*R*,6″*R*) stereochemistry. The ^{13}C NMR spectra (noise-decoupled and INEPT) revealed the C-6″ signal (δ85.66) lowfield to that of C-3″ (δ84.41) and the ^{1}H NMR spectrum displayed the C-6″ proton signal as double-doublets (J = 9, 6 Hz) at δ4.07. These spectral data are consistent with *trans* configurations at the C-3″ and C-6″ chiral centres [6–8]. The findings thus suggest that **1a** is presumably derived in plants from tyrosine bioconversion into *N*-benzoyl-*O*-neryl or *O*-geranyl tyramine [2, 3] via acylation with ATP-activated benzoic acid and alkylation with either neryl or geranyl pyrophosphate. A subsequent enzymatic stereospecific epoxidation of a monoterpenoid moiety produces the hypothetical 2″*S*,3″*R*-6″*S*,7″-diepoxytetrahydroneryl or 2″*S*,3″*S*-6″*R*, 7″-diepoxytetrahydrogeranyl intermediate (**2a** or **2b**). Either one of these is assumed to undergo the regio- and stereospecific nucleophilic acetoxylation ($MeCO_2H$) on the tertiary carbon atom (C-7″). This is followed by spontaneous cyclization with stereochemical inversion at the 3″*R* or 3″*S* chiral centre (C-3″) giving rise to the *trans,threo* (2″*S*,3″*S*,6″*S*) diastereomer (**1a**) from **2a** or the *trans,erythro* (2″*S*,3″*R*,6″*R*) diastereomer, the *N*-benzoyl-4′-[(2″*S*,3″*R*,6″*R*)-7″-acetoxy-2″-hydroxy-3″,7″-dimethyl-3″,6″-epoxyoctyloxy]phenethylamine (**1b**) from **2b** [9].

The assignment of *trans,threo* (2″*S*,3″*S*,6″*S*) stereochemistry to (+)-tuberine (**1a**) in preference to *trans,erythro* (2″*S*,3″*R*,6″*R*) relative configurations (**1b**) was inferred from the ^{1}H NMR spectrum which revealed that the C-2″ proton resonance was shifted downfield considerably (δ4.14). This observation is consistent with the *threo*-alcohol (**1a**), where the carbinol methine proton (C-2″) is in close proximity to the deshielding influence of the oxygen atom of the tetrahydrofuran ring [10] and the C-2″ hydroxyl function further away from it is involved in forming a weak intramolecular hydrogen-bond with the carbonyl oxygen of the amide group, as indicated by the IR spectrum.

The aromatic proton region of the ^{1}H NMR spectrum showed the five-proton A_2BX_2 system of the *N*-benzoyl residue and the four-proton A_2B_2 double-doublet pattern of the *p*-substituted phenyl part of tyramine. The aliphatic proton region of the spectrum exhibited the ethylamide *N*-proton unresolved broad triplet, C-1 two-proton quartet and C-2 two-proton triplet. This region of the ^{1}H NMR spectrum also showed the resonance signals which accounted for the 21 protons of the functionalized monoterpenoid substituent of **1a**. Of these were the four three-proton singlets, three of which showed chemical shift values (δ1.99, 1.46, 1.49) typical of those observed for an α-acetoxy-isopropyl function (C-6″), and the remaining signal at δ1.25 was ascribed to the C-3″ methyl group [11]. The one-proton double-doublets at δ4.07 was assigned to the C-6″ proton, the pronounced downfield shift of which was presumably attributable in part to the deshielding effect of the C-7″ acetoxyl substituent [9, 11]. Irradiation at the C-5″ non-equivalent two one-proton overlapped multiplets (δ1.92) collapsed the 6″-proton double-doublets to a singlet and the C-4″ non-equivalent α- and β-proton multiplets to broadened double-doublets. The downfield shift of the C-4″ α-proton multiplet (δ2.18) relative to that of the C-4″ β-proton (δ1.72) is probably due to its *cis* position with respect to the C-6″ acetoxy-isopropyl group [8].

The C-2″ hydroxyl proton broadened singlet resonated at δ2.6 and disappeared with the signal assigned to the amide *N*-proton on addition of CH_3OD. The apparent upfield shift of this proton signal indicated its close proximity to the shielding current of the *p*-substituted phenyl substituent. This, presumably with the carbonyl oxygen atom of the amide group, serves as a bidentate ligand for this proton (Dreiding model). The three-proton ABX system comprising the C-2″ methine proton (X) and the C-1″ non-equivalent methylene protons (AB) appeared in the 400 MHz ^{1}H NMR spectrum as three pairs of X lines (*t*) arranged at *ca* δ4.14 and a typical AB-type quartet ascribable to an ab subspectrum centred at δ3.95 while the other ab subspectrum degenerated to a deceptively simple singlet (*dss*) resonating at δ3.93 [12]. An iterative computer analysis based on the spin system ABX was carried out to fit the line positions. It furnished the chemical shifts δ_X 4.14, δ_B 3.95 and δ_A 3.93 and coupling constants J_{AB} 7.5 ± 0.6, J_{BX} 10.4 ± 1.1 and J_{AX} −3.7 ± 1.1 Hz.

The proton noise-decoupled ^{13}C NMR and INEPT ^{13}C NMR spectra [13] revealed the 27 carbon resonance signals of **1a** and differentiated the various carbon atom types, to which the respective chemical shifts are assigned as described in the Experimental.

The high-resolution mass spectrum corroborated the other spectral (IR, UV, ^{1}H NMR, ^{13}C NMR) findings. It showed, besides the $[M]^+$ at *m/z* 469, the 7″-acetoxy-2″-hydroxy-3″,7″-dimethyl-3″,6″-epoxyoctyl substituent and the *N*-benzoyltyramine moiety derivable characteristic fragment ions at *m/z* 185, 169, 155, 151, 143, 137, 125, 111, 85, 71, 60 [9, 11] and at *m/z* 241, 122, 120, 107, 105, 91, 77 [2, 3], respectively. Whereas the diagnostically important ions at *m/z* 409, 368, 348, 294, 288, 284, 262 incorporated the C-1″ and C-4′ ethereal oxygen linked fragment ions derivable from the aforementioned two moieties of **1a**.

EXPERIMENTAL

Spectral measurements and determination of microanalyses, optical rotations and mps were carried out as described in a previous paper [1].

Isolation of (+)-*tuberine* (**1a**). Because of similar TLC patterns, fractions 85F–135F [1] were combined and evapd. From the residue, (+)-tuberine (R_f 0.41) was separated preparatively on 25 mm silica gel 60 GF_{254} TLC plates developed with EtOAc–hexane (3:1) and visualized with Dragendorff reagent. Recrystallization of **1a** from EtOAc–petrol afforded pure alkaloid (60 mg).

Determination of the absolute configuration at hydroxylated C-2″ of **1a** [4, 5]. A soln of 112.4 mg of racemic α-phenylbutyric anhydride (3.63×10^{-4} mol) and 36 mg of **1a** (7.68×10^{-5} mol) in 2 ml pyridine was kept at room temp. for 72 hr. Excess anhydride was destroyed by adding 1 ml H_2O and allowing the mixture to stand for 15 hr. Then, after addition of 4 ml H_2O, the soln was extracted with Et_2O (5 × 10 ml), then washed with H_2O (10 ml) and 5% $NaHCO_3$ (3 × 10 ml) and again with H_2O (3 × 10 ml). The combined aq. extracts were washed with $CHCl_3$ (3 × 25 ml) and then acidified with 1 N H_2SO_4 soln. The acidified soln was extracted with $CHCl_3$ (8 × 20 ml) and the $CHCl_3$ extract dried (Na_2SO_4) and after removal of solvent *in vacuo*, afforded 75.84 mg (*R*)-(−)-α-phenylbutyric acid, $[\alpha]_D^{25}$ −0.923° (C_6H_6), which corresponded to an optical yield of 8.1% (−).

(+)-*Tuberine* (**1a**). Colourless needles (60 mg) from EtOAc–petrol, mp 150–152°, $[\alpha]_D^{25}$ + 12.5° (*c* 0.12; $CHCl_3$), MS *m/z* (rel. int.): 469.247 (0.31) $[M]^+$ (Found: C, 69.04, H, 7.60. N, 3.01. Calc. for $C_{27}H_{35}NO_6$: C, 69.05, H, 7.52, N, 2.98%, MW, 469.246), 409 (12) $C_{25}H_{31}NO_4$, 368 (4) $C_{22}H_{26}NO_4$, 348 (13) $C_{20}H_{28}O_5$, 294 (5) $C_{19}H_{20}NO_2$, 288 (52) $C_{18}H_{24}O_3$, 184 (17) $C_{17}H_{18}NO_3$, 241

(4), $C_{15}H_{15}NO_2$, 185 (86) $C_{10}H_{17}O_3$, 169 (22) $C_{10}H_{17}O_2$, 162 (8) $C_{10}H_{10}O_2$, 155 (27) $C_9H_{15}O_2$, 151 (4) $C_{10}H_{15}O$, 143 (29) $C_8H_{15}O_2$, 137 (4) $C_9H_{13}O$, 125 (100) $C_8H_{13}O$, 122 (6) C_7H_8NO, 120 (55) C_8H_8O, 111 (5), $C_7H_{11}O$, 107 (17) C_7H_7O, 105 (76) C_7H_5O, 91 (4) C_7H_7, 85 (4) C_5H_9O, 77 (24) C_6H_5, 71 (24) C_4H_7O, 60 (5) $C_2H_4O_2$. ^{1}H NMR ($CDCl_3$): benzoyl part: δ7.41 (2H, *td*, J = 8, 1 Hz, 3‴-H, 5‴-H), 7.49 (1H, *tt*, J = 8, 1 Hz, 4‴-H), 7.69 (2H, *dd*, J = 8, 1 Hz, 2‴-H, 6‴-H); tyramine moiety: δ2.88 (2H, *t*, J = 7 Hz, 2-H), 3.69 (2H, *q*, J = 7 Hz, 1-H), 6.10 (1H, *br s*, N-H), 6.90 (2H, *d*, J = 9 Hz, 3′-H, 5′-H), 7.16 (2H, *d*, J = 9 Hz, 2′-H, 6′-H); substituted tetrahydrofuran moiety: δ3.93 (1H, *dss*, J = 7.5, −3.7 Hz, 1″-H_A), 3.95 (1H, *q*, J = 7.5, 10.4 Hz, 1″-H_B), 4.14 (1H, *tpl*, J = 10.4, −3.7 Hz, 2″-H_X), 2.60 (1H, *br s*, 2″-OH), 1.25 (3H, *s*, 3″-Me), 1.72 (1H, *m*, 4″-H_β), 2.18 (1H, *m*, 4″-H_α), 1.92 (2H, *m*, 5″-$H_{\alpha,\beta}$), 4.07 (1H, *dd*, J = 9, 6 Hz, 6″-H), 1.46 (3H, *s*, 7″-Me), 1.49 (3H, *s*, 8″-H), 1.99 (3H, *s*, 7″-OOCMe); ^{13}C NMR ($CDCl_3$)O_2CMe: δ22.45 (–Me), 170.40 (O_2C–); benzoyl part: δ167.45 (–NCO–), 134.78 (C-1‴), 129.82 (C-2‴, C-6‴), 126.82 (C-3‴, C-5‴), 131.39 (C-4‴); tyramine moiety: δ41.29 (C-1), 34.85 (C-2), 134.78 (C-1′), 128.58 (C-2′, C-6′), 114.97 (C-3′, C-5′), 157.52 (C-4′); substituted tetrahydrofuran moiety: δ69.27 (C-1″), 75.13 (C-2″),84.41 (C-3″), 33.82 (C-4″), 26.60 (C-5″), 85.66 (C-6″), (82.51 (C-7″), 22.32 (C-8″), 21.94 (C-7″-Me), 22.86 (C-3″-Me); UV λ_{max}^{EtOH} nm (log ε): 226 (4.39), 274 (3.44), 283 (3.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 3440, 1720, 1650, 1250.

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