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J. Nat. Prod., **1992**, 55 (7), 899-903• DOI:
10.1021/np50085a008 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 7, pp. 899-903, July 1992

NEW ALKALOIDS FROM *HAPLOPHYLLUM TUBERCULATUM*

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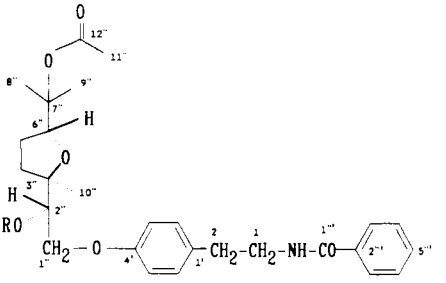
ABSTRACT.—Two new alkaloids, tubacetine [**3**] and tubasenecine [**4**], were isolated from the aerial parts of *Haplophyllum tuberculatum* (Rutaceae). Their identities were established from nmr data, including a study of the 2D INADEQUATE spectra of the monoterpene part of **3**. In addition, the alkaloid 7-hydroxy-8-(3-methyl-2-butenyl)-4-methoxyfuro[2,3*b*]quinoline [**5**] has been identified in this source for the first time.

Haplophyllum tuberculatum (Forssk.) A. Juss. (Rutaceae) grows throughout Saudi Arabia, especially on fallow land and sandy soil. The flowering and fruiting branches of this plant are used by the public for a variety of ailments, including malaria, rheumatoid arthritis, and gynecological troubles (1). A number of constituents have been reported to occur in this plant; these include the lignans justicidin A and justicidin B (2), dyphyllin (2) and tuberculatin (2), and the quinoline alkaloids dihydroperfamine (3), 3-dimethylallyl-4-dimethylallyloxy-2-quinolone (4), evoxine (5), γ-fagarine (5), flindersine (4), folifine (6), haplofoline (6), and skimmianine (7). The amide tuberine [**1**] is the only tyramine alkaloid that has so far been reported (8) in this plant. This paper describes the isolation and structure elucidation of two related compounds, now named tubacetine [**3**] and tubasenecine [**4**]. It also reports, for the first time, the presence of 7-hydroxy-8-(3-methyl-2-butenyl)-4-methoxyfuro[2,3*b*]quinoline [**5**] (10) in this source.



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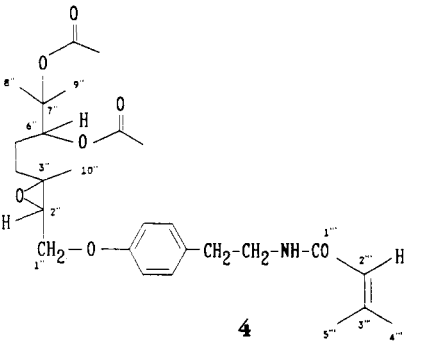
2 R=Ac



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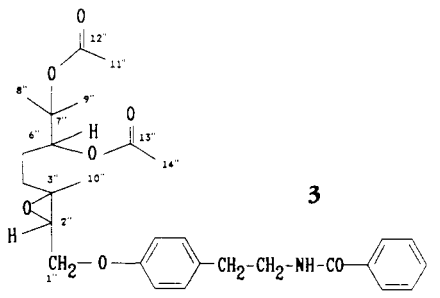

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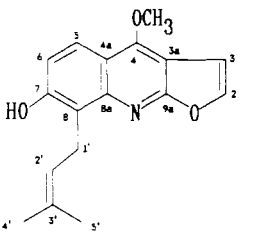

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The CH_2Cl_2 extract of the aerial parts of *H. tuberculatum* was partitioned between *n*-hexane and MeCN. Tubacetine [**3**] was isolated from the MeCN fraction by repeated flash chromatography (11), initially using Me_2CO-C_6H_6 (1:19) as an eluent followed by 25% Me_2CO in *n*-hexane. Compound **3** was obtained as a colorless microcrystalline solid, $C_{29}H_{37}O_7N$, mp 71.7–72.7°, $[\alpha]^{22}D$ +12° (c = 0.1, $CHCl_3$). The ^{1}H-nmr spectrum clearly suggested that the compound was closely related to tuberine [**1**], which was previously reported (8) in the same plant. In fact, the tyramine portion of the molecule with its AA′ BB′ system and benzamide residue was almost indistinguishable from that of tuberine [**1**] (8) (see Table 1). The possibility that the compound was acetyltuberine [**2**], a structural isomer, was quickly ruled out by direct comparison. Their nmr spectra were distinctly similar, but not identical. Thorough nmr analysis of the signals due to the terpene portion of the molecule (C-1″ through C-10″) using 2D nmr techniques including COSY, HETCOR, and INADEQUATE, unambiguously established the carbon-carbon connectivities in this moiety. However, the connectivity between C-2″ and C-3″ could not be directly established due to the proximity of their field positions, 60.4 and 60.2 ppm, respectively. Nevertheless, their connectivity could be easily inferred from their field positions, which suggested an epoxide function (12), whose presence was also substantiated from the molecular formula. The absolute stereochemistry at the three chiral centers of 3, namely, 2″, 3″, and 6″, is yet to be determined.

The aerial parts of *H. tuberculatum*, collected in the central region of Saudi Arabia, yielded, in addition to **3**, the related compound tubasenecine [**4**]; **4** could not be detected in other collections of the plant. The nmr data of **4** (see Table 1) were almost identical to those of **3**, except for the absence of the benzoic acid residue and the presence, instead, of a senecioic acid moiety. The presence of the latter was confirmed by the presence in the ^{1}H-nmr spectrum of a pair of three-proton doublets at δ 2.13 (J = 1.0 Hz) and 1.81 (J = 1.0 Hz) due to H-5‴ and H-4‴, respectively (13), and a broad singlet at δ 5.47 due to H-2‴ (13,14).

The structures of both tubacetine [**3**] and tubasenecine [**4**] are closely related to that of acidissimin (15), a tyramine derivative isolated from the fruits of *Limonia acidissima* (Rutaceae). The latter, however, has an ester group that was placed on C-4″, instead of the biogenetically favored C-6″.

Another chromatographic fraction obtained in the course of isolation of **3** and **4** yielded the alkaloid 7-hydroxy-8-(3-methyl-2-butenyl)-4-methoxyfuro[2,3*b*]quinoline [**5**], hitherto unreported in *H. tuberculatum*. Compound **5** has not been previously reported in the genus *Haplophyllum* and was reported (10) to occur in the leaves of *Sarcomelicope glauca*, another member of the Rutaceae. The identity of **5** was based on comparison of its physical and spectral data with those reported by Mitaku *et al.* (10). In addition, the ^{13}C-nmr assignments of **5** are reported here for the first time. Compound **5** has been detected in only one collection of *H. tuberculatum*, and this was the same collection that also yielded **4**.

Tuberine [**1**] has been reported (16) to possess potent antimicrobial activity against a variety of Gram positive organisms, including *Staphylococcus aureus*. However, in our hands, neither tuberine [**1**], nor any of the compounds isolated in the present study, was found to have any antimicrobial activity below 100 μg/ml. The conditions used for antimicrobial testing were similar to those previously reported (17).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were determined on a Gallen-Kamp mp apparatus or a Mettler 9100 electrothermal unit, and are uncorrected. Ir spectra were obtained on a Pye Unicam ir spectrophotometer, model SP3-300, while uv spectra were recorded on a Varian uv-visible spec-

TABLE 1. ^{1}H and ^{13}C Nmr Assignments for Tubacetine [3] and Tubasenecine [4].

Position	Compound			
	3		4	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1	3.68 q (6.0) reduced to t upon D_2O exchange	41.3 (2)	3.50 q (6.8) reduced to t upon D_2O exchange	40.4 (2)
2	2.87 t (6.8)	34.8 (2)	2.76 t (7.0)	34.9 (2)
1′	—	131.5 (0)[c]	—	131.7 (0)
2′,6′	7.16 d (8.6)	129.9 (1)	7.11 d (8.6)	129.8 (1)
3′,5′	6.89 d (8.6)	114.9 (1)	6.87 d (8.6)	114.8 (1)
4′	—	157.3 (0)	—	157.2 (0)
1″	4.09 ddd (4.6,5.7,10.9)	66.9 (2)	4.08 ddd (4.7,5.8,11.0)	66.9 (2)
2″	3.15 overlap, dd (~5.1,5.1)	60.4 (1)	3.13 overlap, dd (~5.3,5.3)	60.4 (1)
3″	—	60.2 (0)	—	60.3 (0)
4″	1.6 m	34.5 (2)	1.6 m	34.5 (2)
5″	1.6 m	24.4 (2)	1.6 m	24.4 (2)
6″	5.12 dd (2.5,9.6)	76.9 (1)	5.12 dd (2.5,9.7)	77.0 (1)
7″	—	82.5 (0)	—	82.5 (0)
8″,9″	1.48 s and 1.44 s	22.4 (3)[d], 22.2 (3)[d]	1.48 s and 1.44 s	22.4 (3)[d], 22.2 (3)[d]
10″	1.33 s	17.1 (3)	1.33 s	17.1 (3)
11″,14″	2.09 s, 1.96 s	22.1 (3)[d], 20.9 (3)[d]	2.09 s and 1.96 s	22.1 (3)[d], 20.9 (3)[d]
12″,13″	—	170.5 (0)[e], 170.1 (0)[e]	—	170.5 (0)[e], 170.1 (0)[e]
1‴	—	167.5 (0)	—	167.0 (0)
2‴	—	134.7 (0)	5.47 br s	118.5 (1)
3‴	7.69 br d (6.9)	126.8 (1)	—	150.9 (0)
4‴	7.40 br d (7.0)	128.6 (1)	1.81 d (1.1)[f]	27.1 (3)
5‴	7.48 br t (7.2)	131.4 (1)	2.13 d (1.1)	19.8 (3)
6‴	same as 4‴	same as 4‴	—	—
7‴	same as 3‴	same as 3‴	—	—
-NH	6.17 br t (5.5); exchangeable	—	5.39 br t (5.5); exchangeable	—

[a]The numbers in parentheses represent *J* values and are in Hz.
[b]The numbers in parentheses represent the number of attached protons.
[c]Assignments are confirmed by LR-HETCOR.
[d,e]Assignments in the same column with the same superscript are interchangeable.
[f]See Topcu *et al.* (13) for all assignments of senecioic acid.

trophotometer, model DMS90. Specific rotations were measured on a Perkin-Elmer 241 MC instrument, and low resolution eims and cims spectra were obtained using an E.I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system or an E.I. Finnigan model 4600 quadrupole system. Nmr spectra were determined on a Varian VXR-300 spectrometer at 300 and 75 MHz for ^{1}H nmr and ^{13}C nmr, respectively, and chemical shift values are given in δ (ppm) with TMS as internal standard. Standard Varian pulse sequences were used for APT, DEPTGL, COSY, HETCOR (including long range), and INADEQUATE spectra, which aided nmr assignments. Tlc analyses were performed on si gel G plates using C_6H_6-Me_2CO (19:1) as a solvent unless otherwise specified, and the chromatograms were visualized under short wavelength uv light (254 nm) or by spraying with Dragendorff's reagent (19).

PLANT MATERIAL.—The aerial parts of *H. tuberculatum* were collected in April 1987, near the Riyadh-Gassim highway, 100 miles from Riyadh, in central Saudi Arabia (collection A) and in May 1990 in Skaka, in the northern region (collection B). The plant material was identified by Dr. Sultanul Abidin of the Research Center for Medicinal, Aromatic and Poisonous Plants, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, and voucher specimens are being kept at the Center.

ISOLATION OF TUBACETINE [**3**], TUBASENECINE [**4**], AND **5**.—The plant material (collection A, 2 kg) was extracted with CH_2Cl_2 in a Soxhlet for 72 h. The extract (58 g) was partitioned between *n*-hexane and MeCN, 4 × 300 ml of each. The two solvents were presaturated with each other. The MeCN phase was evaporated in vacuo to yield 26 g of a dark green oily residue that contained several alkaloids when analyzed by tlc. Flash chromatography of this residue (13 g) on Si gel using Me_2CO-C_6H_6 (1:19) gave a number of fractions. Fraction A yielded upon purification the previously reported (9) alkaloid buchapine, while fraction B (1.4 g), eluted next, was flash chromatographed on Si gel using C_6H_6-Me_2CO (98:2) to yield 0.22 g of **5**: yellowish crystals from C_6H_6; R_f 0.43; mp 106–108° [lit. (10) not reported]; ^{1}H-nmr, ir, uv, and ms data indistinguishable from those reported (10); ^{13}C nmr ($CDCl_3$) δ 142.5 (C-2), 104.8 (C-3), 101.3 (C-3a) (18), 157.2 (C-4), 113.7 (C-4a) (18), 121.5 (C-5), 115.9 (C-6), 155.8 (C-7), 119.0 (C-8), 145.6 (C-8a), 164.0 (C-9a), 58.9 (OMe), 23.9 (C-1′), 122.3 (C-2′), 135.2 (C-3′), 25.8 (C-4′, corresponding to the Me at δ 1.78) and 18.1 (C-5′, corresponding to the Me at δ 1.91) (10). Fractions C–F were eluted in this order, of which C contained dihydroperfamine (3), while E was crude tuberine [**1**] (8) and F was mostly flindersine (4). Tubacetine [**3**] and tubasenecine [**4**] were contained in fraction D, R_f values 0.27 and 0.18, respectively, using 30% Me_2CO in C_6H_6. Flash chromatography of this fraction (2.5 g) on Si gel using Me_2CO–*n*-hexane (1:3) as solvent yielded 0.546 g of **3** as a microcrystalline solid from Et_2O/*n*-hexane: mp 71.7–72.7; $[\alpha]^{22}D$ +12° (*c* = 0.1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3380 (NH), 1740 (*CO*Me) and 1640 (NH*CO*); uv λ max (MeOH) nm (log ε) 279 (3.27), 273 (3.41) and 220 (4.47); ^{1}H and ^{13}C nmr see Table 1; cims (isobutane) *m/z* (% rel. int.) $[M+1]^+$ 512 (65) with the base peak at *m/z* 392. *Anal.* calcd for $C_{29}H_{37}O_7N$: C 68.08, H 9.29, N 2.74; found C 68.10, H 9.31, N 2.77.

Tubasenecine [**4**] (110 mg) was eluted next and was obtained as a faint yellow gum: $[\alpha]^{25}D$ +12° (*c* = 0.1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3300 (NH), 1730 (-*CO*Me) and 1645 (-*CO*NH); uv λ max (MeOH) nm (log ε) 281 (3.10), 274 (3.21), 218 (4.42); ^{1}H and ^{13}C nmr see Table 1; cims (isobutane) *m/z* (% rel. int.) $[M+1]^+$ 490 (28) with the base peak at *m/z* 390. *Anal.* calcd for $C_{27}H_{39}O_7N$: C 66.23, H 8.03, N 2.86; found C 66.32, H 8.12, N 2.91.

Similar processing of 2 kg of collection B of the plant material yielded 0.94 g of **3**. Both **4** and **5** were absent.

ACETYLATION OF TUBERINE [**1**].—Tuberine [**1**] (100 mg) was dissolved in pyridine (2 ml) and Ac_2O (2 ml). The solution was kept overnight at room temperature and worked up by adding 40 ml of 10% aqueous $NaHCO_3$ and stirring for 15 min. The mixture was then diluted with H_2O (80 ml) and extracted with $CHCl_3$ (4 × 160 ml). The combined $CHCl_3$ phases were washed with 5% HCl (4 × 80 ml), H_2O (80 ml), 5% aqueous $NaHCO_3$ (80 ml), and H_2O again (80 ml). Drying (anhydrous Na_2SO_4) and evaporation yielded 90 mg of crude acetyltuberine [**2**] that was further purified by flash chromatography on Si gel using 6% Me_2CO in C_6H_6 as a solvent. The product (80 mg) was obtained as a colorless gum: $[\alpha]^{25}D$ +3° (*c* = 0.1, $CHCl_3$); ir ν max (neat) cm^{-1} 3340 (NH), 1730 (*CO*Me), 1640 (*CO*NH); uv λ max (MeOH) nm (log ε) 280 (3.35), 273 (3.50), 220 (4.54); ^{1}H nmr ($CDCl_3$) 3.67 (2H, q, *J* = 6.1 Hz, H-1), 2.86 (2H, t, *J* = 7.0 Hz, H-2), 7.13 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.85 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 4.04 (2H, m, H-1″), 5.28 (1H, dd, *J* = 2.5 and 7.5 Hz, H-2″), 1.9 (4H, m, H-4″ and H-5″), 4.29 (1H, dd, *J* = 2.6 and 10.6 Hz, H-6″), 1.43 and 1.46 (3H each, s, H-8″ and H-9″, unassigned), 1.25 (3H, s, H-10″), 1.96 and 2.08 (3H each, s, H-11″ and H-14″, unassigned), 7.69 (2H, brd, *J* = 6.8 Hz, H-3‴ and H-7‴), 7.39 (2H, brt, *J* = 6.8 Hz, H-4‴ and H-6‴), 7.47 (1H, brt, *J* = 6.8 Hz, H-5‴), 6.22 (1H, brt, *J* = 4.6 Hz, NH); eims *m/z* (% rel. int.) $[M]^+$ 511 (<1%) with the base peak at *m/z* 244; ^{13}C nmr ($CDCl_3$) δ 41.3 (C-1), 35.0 (C-2), 131.2 (C-1′), 129.7 (C-2′ and C-6′), 114.9 (C-3′ and C-5′), 157.3 (C-4′), 67.3 (C-1″), 75.5 (C-2″), 83.1 (C-3″ or C-7″), 34.7 (C-4″), 26.2 (C-5″), 85.2 (C-6″), 82.4 (C-7″ or C-3″), 22.8, 22.4, 22.3, 21.7, and 21.1 (2 acetate methyls, C-8″, C-9″ and C-10″, unassigned), 170.4 and

170.3 (2 Me*C*OO, unassigned), 167.4 (C-1‴), 134.7 (C-2‴), 126.8 (C-3‴, C-7‴), 128.5 (C-4‴, C-6‴), 131.3 (C-5‴). Assignments of C-1 and C-2 of tuberine [**1**] were reversed in Sheriha *et al.* (20) and were inadvertently left uncorrected in McPhail *et al.* (8). Also, assignments of carbons 1′ and 2‴ in tuberine [**1**] and acetyltuberine [**2**] can now be differentiated by reference to their assignments in tubacetine [**3**], which were confirmed by long range HETCOR, and also by the absence of the signal at δ 134.7 due to C-2‴ in tubasenecine [**4**].

LITERATURE CITED

1. A.M. Ageel, J.S. Mossa, M. Tariq, M.A. Al-Yahya, and M.S. Al-Said, "Saudi Plants Used in Folk Medicine," Department of Scientific Research, King Abdel-Aziz City for Science and Technology (KACST), Riyadh, Saudi Arabia, 1987, p. 211.
2. G.M. Sheriha and K.M. Abou-Amer, *Phytochemistry*, **23**, 151 (1984).
3. E.A. El-Kashoury, M.A Abdel-Kawy, A.M. El-Fishawy, and F.M. Soliman, *Bull. Fac. Pharm. Cairo Univ.*, **29**, 1 (1991).
4. I. Mester, *Fitoterapia*, **44**, 123 (1973).
5. A. Al-Shamma, N.A. Al-Douri, and J.D. Phillipson, *Phytochemistry*, **18**, 1417 (1979).
6. Y. Rashkes, Z.S. Faizutdinova, and S. Yunusov, *Khim. Prir. Soedin.*, **6**, 107 (1970).
7. S.A. Khalid and P.G. Waterman, *Planta Med.*, **43**, 148 (1981).
8. A.T. McPhail, D.R. McPhail, M.S. Al-Said, M.M. El-Domiaty, and F.S. El-Feraly, *Phytochemistry*, **29**, 3055 (1990), and references cited therein.
9. G.M. Sheriha, K. Abouamer, B.Z. Elshtaiwi, A.S. Ashour, F.A. Abed, and H.H. Alhallaq, *Phytochemistry*, **26**, 3339 (1987).
10. S. Mitaku, A. Skaltsounis, F. Tillequin, M. Koch, J. Pusset, and G. Chauviere, *J. Nat. Prod.*, **49**, 1091 (1986).
11. W.C. Still, M. Khan, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
12. J.S Mossa, M.M. Al-Yahya, M.S. Hifnawy, A.A. Shehata, F.S. El-Feraly, C.D. Hufford, D.R. McPhail, and A.T. McPhail, *Phytochemistry*, **29**, 1595 (1990).
13. G. Topcu, G.A. Cordell, N.R. Farnsworth, and H.S. Fong, *J. Pharm. Sci.*, **77**, 553 (1988).
14. A. Ulubelen, *Phytochemistry*, **23**, 2123 (1984).
15. P. Ghosh, P. Sil, S. Das, S. Thakur, W.C. M.C. Kokke, T. Akihisa, N. Shimizu, T. Tamura, and T. Matsumoto, *J. Nat. Prod.*, **54**, 1389 (1991).
16. S.O. Gnan and G.M. Sheriha, *J. Food Prot.*, **49**, 340 (1986).
17. A.M. Clark, F.S. El-Feraly, and W. Li, *J. Pharm. Sci.*, **70**, 951 (1981).
18. J. Pusset, J.L. Lopez, M. Pais, M. Al-Neirabeyeh, and J. Veillon, *Planta Med.*, **57**, 153 (1991).
19. R.J. Grilter, J.M. Bobbitt, and A.E. Schwarting, "Introducton to Chromatography," 2nd ed., Holden-Day, Oakland, California, 1985, p. 134.
20. G.M. Sheriha, K. Abouamer, and B.Z. Elshtaiwi, *Phytochemistry*, **24**, 884 (1985).

Received 6 November 1991