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Thalifaurine and Dehydrodiscretine, New Quaternary Protoberberines from *Thalictrum fauriei*

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Abstract □ Two new quaternary protoberberines, thalifaurine and dehydrodiscretine, were isolated from the ethanolic extract of *Thalictrum fauriei* Hayata. Based on spectral analysis and correlation with compounds of known structure, thalifaurine was shown to be 3-hydroxy-2-methoxy-10,11-methylenedioxyberbinium chloride and dehydrodiscretine was established as 3-hydroxy-2,10,11-trimethoxyberbinium chloride. The structural assignment was confirmed by synthesis of both compounds. Magnoflorine also was isolated and identified.

Keyphrases □ Thalifaurine—new quaternary protoberberine isolated from *Thalictrum fauriei*, structural determination □ Dehydrodiscretine—new quaternary protoberberine isolated from *Thalictrum fauriei*, structural determination □ Protoberberines, quaternary—thalifaurine and dehydrodiscretine, isolation from *Thalictrum fauriei*, structural determination □ *Thalictrum fauriei*—isolation of thalifaurine and dehydrodiscretine and their structural determination

Thalictrum fauriei Hayata (Ranunculaceae) is a perennial herb that is distributed widely over the mountainous area of Taiwan, especially in wet places (1). In view of an increasing interest in the biological activity of some Thalictrum alkaloids (2), a study was initiated on the alkaloidal constituents of *T. fauriei*.

Previous reports from this laboratory described the isolation and characterization of three known aporphines, (+)-oconovine (Ia) (3), (+)-isocorydine (Ib) (3), and (+)-corydine[1] (Ic), from the tertiary base fraction. Continuing investigation of the quaternary base fraction has provided two new protoberberinium salts, thalifaurine (IIa) and dehydrodiscretine (IIb), in addition to the known aporphine magnoflorine (III). The isolation and structural characterization of these quaternary salts are described in this report.

[1] C.-H. Chen, T.-M. Chen, and C. Lee, unpublished data.

RESULTS AND DISCUSSION

The ethanolic extract of the whole plant material of *T. fauriei* was processed by the usual acid–base treatment and solvent partitioning to separate the quaternary alkaloid fraction from the tertiary base fraction. The quaternary salt was precipitated with Mayer reagent and then was exchanged by an anionic resin into the chloride form. Extensive fractionation of this chloride salt on silica gel columns yielded three crystalline compounds, designated as alkaloids Q-3, Q-4, and Q-6 according to their increasing polarity on TLC plates.

Alkaloid Q-4—Alkaloid Q-4 was isolated as the chloride salt, mp 258–260°, and was optically inactive. The UV spectrum appeared as a complicated pattern with absorption maxima at 241, 263, 291, and 341 nm and shoulders at 310 and 380 nm. This pattern was characteristic of quaternary protoberberine salts, and the shoulder at 310 nm suggested a 2,3,10,11-substituted pseudoprotoberberinium pattern (4). Bathochromic shifts in the presence of bases and the broad IR absorption bands at 3510 and 3326 cm^{-1} indicated the phenolic nature of Q-4. The NMR spectrum of Q-4 revealed one methoxy group at δ 4.02, one methylenedioxy group at δ 6.33, two triplets of two protons each at δ 3.17 and 4.74 (J = 6 Hz), and six aromatic protons at δ 6.87 (s, 1H), 7.51 (s, 2H), 7.64 (s, 1H), 8.59 (s, 1H), and 9.25 (s, 1H) ppm.

Simanek *et al.* (5) made a detailed analysis of the chemical shifts for each aromatic proton on various protoberberinium and pseudoprotoberberinium salts. The data for pseudopalmatinium (IIe) and pseudoepiberberinium (IIf) salts were taken from their report and compared with those of Q-4 (Table I); the data for Q-4 were consistent with the 2,3,10,11-substitution pattern. In the chemical-ionization mass spectrum of Q-4 with isobutane as the reagent gas, the molecular ion M^+ appeared at m/e 322 and base ion c appeared at m/e 320; both were highly abundant, which reflected the unusual stability of the parent ion under the chemical-ionization mode. Ion a at m/e 323 and ion b at m/e 337 represented the result from thermal disproportionation of the parent ion (M^+), a fact proven for berberine derivatives by Habermehl *et al.* (6). Ions d (m/e 177), e (m/e 178), and f (m/e 176) suggested the presence of hydroxy and methoxy groups on ring A. Ion g (m/e 148) served to locate the methylenedioxy group on ring D.

Two structures were compatible with the evidence: 3-hydroxy-2-methoxy-10,11-methylenedioxyberbinium chloride (IIa) and 2-hydroxy-3-methoxy-10,11-methylenedioxyberbinium chloride (IIc).

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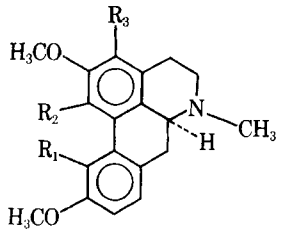
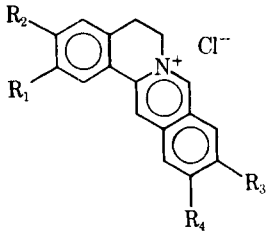


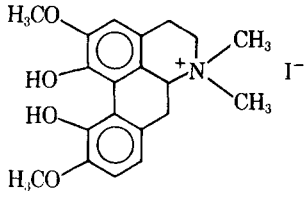
I

Ia: $R_1 = OH$, $R_2 = R_3 = OCH_3$
Ib: $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$
Ic: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$

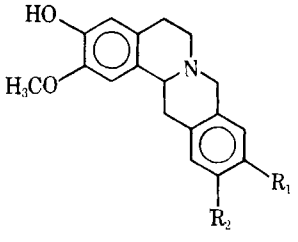


II

IIa: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = R_4 = OCH_2O$
IIb: $R_1 = R_3 = R_4 = OCH_3$, $R_2 = OH$
IIc: $R_1 = OH$, $R_2 = OCH_3$, $R_3 = R_4 = OCH_2O$
IId: $R_1 = OH$, $R_2 = R_3 = R_4 = OCH_3$

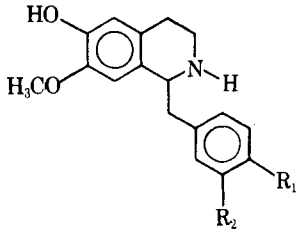


III (as iodide)



IV

IVa: $R_1 = R_2 = OCH_2O$
IVb: $R_1 = R_2 = OCH_3$



V

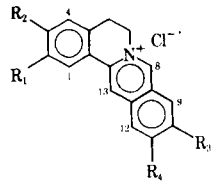
Va: $R_1 = R_2 = OCH_2O$
Vb: $R_1 = R_2 = OCH_3$

Structure IIc is dehydropseudocheilanthifoline, a naturally occurring compound isolated from *Isopyrum thalictroides* (7). An authentic sample of IIc was different from Q-4 in all respects. Thus, Structure IIa was assigned tentatively to Q-4.

On reduction with sodium borohydride, Q-4 yielded a tetrahydroprotoberberine, Q-4-H, mp 142–144°. High-resolution mass spectral analysis of the molecular ion at *m/e* 325 agreed with a composition of $C_{19}H_{19}NO_4$. The UV absorption maxima at 230 and 290 nm were consistent with the expected chromophore, and a bathochromic shift on base treatment coupled with the IR bands at 3571 cm^{-1} suggested a phenolic function. The NMR spectrum showed one methoxy group at δ 3.85, one methylenedioxy group at δ 5.86, and four aromatic protons at δ 6.56 (s, 2H), 6.65 (s, 1H), and 6.82 (s, 1H) ppm. The electron-impact mass spectrum revealed the collapse of ring C to give base peak k (*m/e* 148) from ring D and ions i (*m/e* 178) and j (*m/e* 176) from rings A and B.

These data were identical to those of an authentic sample of 3-hydroxy-2-methoxy-10,11-methylenedioxyberbine (IVa), a by-product obtained from Suguna and Pai (8) in their synthesis of tetrahydrogroenlandicine. By a standard procedure, 6-hydroxy-7-methoxy-1-(3′,4′-methylenedioxybenzyl)-1,2,3,4-tetrahydroisoquinoline (Va) was synthesized and condensed with formaldehyde to give IVa, which also was found to be identical to Q-4-H. Further oxidation of IVa with mer-

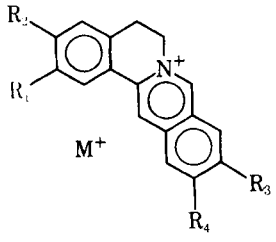
Table I—Comparison of the NMR Data of Q-4 and Q-3 with Pseudoprotoberberinium Salts IIe and IIf in the Aromatic Region



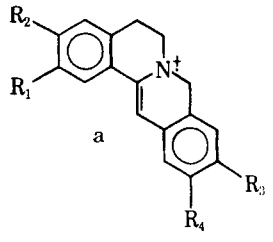
IIa: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = R_4 = OCH_2O$
IIb: $R_1 = R_3 = R_4 = OCH_3$, $R_2 = OH$
IIe: $R_1 = R_2 = R_3 = R_4 = OCH_3$
IIf: $R_1 = R_2 = OCH_3$, $R_3 = R_4 = OCH_2O$

Compound	Proton H-4	H-12	H-1	H-9	H-13	H-8	Solvent
IIe[a]	7.07	7.60	7.63	7.67	8.83	9.50	Dimethyl sulfoxide-d_6
IIf[a]	7.07	7.55	7.62	7.67	8.80	9.47	Dimethyl sulfoxide-d_6
IIa (Q-4)	6.87	7.51	7.51	7.64	8.59	9.25	CD_3OD
IIb (Q-3)	6.53	7.33	7.43	7.43	8.27	9.07	CD_3OD

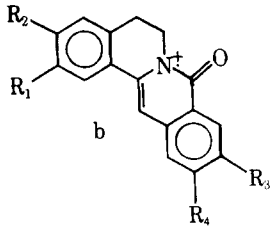
[a] Values for IIe and IIf were taken from Ref. 5.



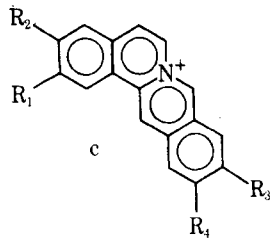
IIa: *m/e* 322 (93.8%)
IIb: *m/e* 338 (0.3%)



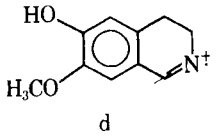
IIa: *m/e* 323 (40.3%)
IIb: *m/e* 339 (0.3%)



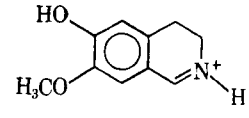
IIa: *m/e* 337 (11.8%)
IIb: *m/e* 353 (2.0%)



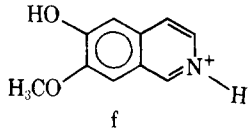
IIa: *m/e* 320 (100%)



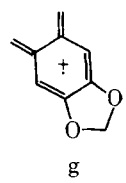
IIa: *m/e* 177 (1.8%)
IIb: *m/e* 177 (1.1%)



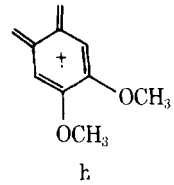
IIa: *m/e* 178 (1.8%)
IIb: *m/e* 178 (1.7%)



IIa: *m/e* 176 (2.7%)

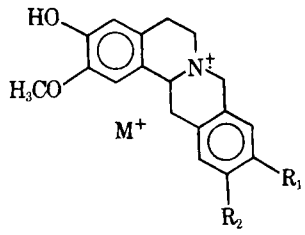


IIa: *m/e* 148 (4.0%)

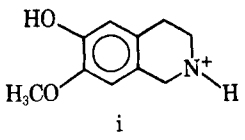


IIb: *m/e* 164 (3.0%)

Chemical-ionization mass spectral fragmentation of Q-4 (IIa) ($R_1 = OCH_3$, $R_2 = OH$, $R_3 = R_4 = OCH_2O$) and Q-3 (IIb) ($R_1 = R_3 = R_4 = OCH_3$, $R_2 = OH$) with isobutane as the reagent gas.

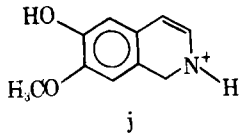


IV*a*: *m/e* 325 (62.5%)
IV*b*: *m/e* 341 (7.8%)



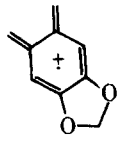
i

IV*a*: *m/e* 178 (1.2%)
IV*b*: *m/e* 178 (2.8%)



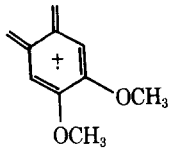
j

IV*a*: *m/e* 176 (29.2%)
IV*b*: *m/e* 176 (12.0%)



k

IV*a*: *m/e* 148 (100%)



l

IV*b*: *m/e* 164 (100%)

Major fragments from electron-impact mass spectra of Q-4-H (IV*a*) ($R_1 = R_2 = OCH_2O$) and Q-3-H (IV*b*) ($R_1 = R_2 = OCH_3$).

curic acetate provided 3-hydroxy-2-methoxy-10,11-methylenedioxyberbinium chloride (II*a*), which was identical in every respect to Q-4. The structure of Q-4 therefore was firmly established as II*a*, and, since it was a new protoberberine from nature, it was named thalifaurine.

Alkaloid Q-3—Alkaloid Q-3 was isolated in a minute amount as the chloride salt, mp 230–234°, and was optically inactive. The UV spectrum was similar to that of Q-4, showing maximum absorptions at 243, 264.5, 289, 310 (sh), 341, and 379 (sh) nm. A bathochromic shift on base treatment and the IR absorption at 3400 cm^{-1} suggested its phenolic nature. The NMR spectrum showed three methoxy groups at δ 4.07, 4.01, and 3.89 ppm and one triplet of two protons at δ 3.05 ppm (J = 6 Hz), with a similar triplet obscured by the solvent peak. In the aromatic region, six protons were observed at δ 9.07 (s, 1H), 8.27 (s, 1H), 7.43 (s, 2H), 7.33 (s, 1H), and 6.53 (s, 1H) ppm. A comparison in this region with Q-4, II*e*, and II*f* (Table I) suggested that Q-3 is another 2,3,10,11-substituted pseudoprotoberberinium salt.

Examination of its chemical-ionization mass spectrum revealed the molecular ion M^+ (*m/e* 338) and the ions from thermal disproportionation of M^+, a (*m/e* 339) and b (*m/e* 353), all of which supported the skeleton of the parent ion, although they were in significantly lower abundance than similar ions from Q-4. Ions d (*m/e* 177), e (*m/e* 178), and h (*m/e* 164) were in agreement with the presence of three methoxy groups and located the phenolic function on ring A as well.

Two alternative structures were compatible with these data: 3-hydroxy-2,10,11-trimethoxyberbinium chloride (II*b*) and 2-hydroxy-3,10,11-trimethoxyberbinium chloride (II*d*). Structure II*d* is pseudocolumbamine, an alkaloid isolated from *I. thalictroides* (7). An authentic sample of II*d* was entirely different from Q-3, which required the assignment of Structure II*b* to Q-3.

Further evidence came from the reduction of Q-3 with sodium borohydride, giving a tetrahydroprotoberberine, Q-3-H, mp 180–182°. The molecular ion at *m/e* 341 was analyzed for $C_{20}H_{23}NO_4$ by high-resolution mass spectrometry. In agreement with the expected chromophore, the UV spectrum showed maximum absorption at 225 (sh) and 286.5 nm. A bathochromic shift was observed in the presence of base, which corroborated the IR phenolic absorption bands at 3570 and 3440 cm^{-1}. The NMR spectrum revealed three methoxy groups at δ 3.86 (s, 6H), and 3.89 (s, 3H) ppm and four aromatic protons at δ 6.58 (s, 1H), 6.66 (s, 2H), and 6.72 (s, 1H) ppm. The major mode of fragmentation in the electron-impact mass spectrum was the cyclic collapse of ring C, yielding base peak l (*m/e* 164) from ring D and ions i (*m/e* 178) and j (*m/e* 176) from rings A and B.

These spectral data were in good agreement with those of discretine (IV*b*) isolated from *Xylopia discreta* (9). Following literature procedures (10, 11), IV*b* was synthesized by condensing 6-hydroxy-7-methoxy-1-(3′,4′-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (V*b*) with formaldehyde. This synthetic sample of (±)-discretine (IV*b*) was identical with Q-3-H in all respects. In addition, oxidation of IV*b* with mercuric acetate produced a compound, II*b*, that was identical to Q-3. Therefore, the structure of Q-3 was proven to be 3-hydroxy-2,10,11-trimethoxyberbinium chloride (II*b*). Since it is a dehydro form of discretine, it was named dehydrodiscretine.

Alkaloid Q-6—The major alkaloid in the quaternary base fraction was Q-6, isolated as the iodide salt, mp 250–252°. The spectral properties of this compound were identical to those of an authentic sample of magnoflorine (III) iodide.

EXPERIMENTAL[2]

Plant Material—The whole plant of *T. fauriei* Hayata[3] was collected during spring 1976 from the mountains near Wulai, Taipei County, Taiwan. The whole plant material was dried in an oven at 45° and milled to a fine powder.

Extraction and Initial Fractionation—Powdered plant material (3.29 kg) was extracted with 95% ethanol by percolation at room temperature until a negative test for alkaloids in the extract was shown with Valser reagent. The percolate was concentrated *in vacuo* to give 350 g of a greenish-brown semisolid residue. This residue was triturated with 3% citric acid and filtered. The process was continued until the filtrate showed a negative Valser test.

The combined acidic solution (3.5 liters) was extracted with ethyl acetate (4 liters) to remove the acidic and neutral fractions. The citric acid solution was adjusted to pH 9–10 with concentrated ammonium hydroxide and then extracted successively with ether (2 liters) and chloroform (3 liters), which removed the tertiary base fraction. The aqueous solution was adjusted to pH 4.4 with saturated citric acid solution, and Mayer reagent was added to precipitate the quaternary alkaloids.

After standing overnight in a refrigerator, the precipitate was filtered and washed with ether. The residue (106 g) then was dissolved in methanol–acetone–water (3:1:1), and the insoluble material was filtered off. The filtrate was stirred with an anion-exchange resin[4] for 3 days. After removal of the resin by filtration, the filtrate was evaporated under reduced pressure to give a semisolid residue (27.5 g) of the quaternary alkaloids as the chloride salts. Part of this residue (6.6 g) was chromatographed on a silica gel[5] (313 g) column and eluted with methanol in chloroform, with the methanol concentration increasing stepwise (1, 3, 7.5, 15, 30, 50, and 100%). Each 100 ml of eluate was collected and analyzed by TLC.

Isolation and Identification of Thalifaurine (II*a*, Q-4)—The residue (260 mg) from column fractions 50–52, eluted with 30% methanol in chloroform, was crystallized from methanol and recrystallized from the same solvent, yielding Q-4 as fine yellow needles (50 mg), mp 258–260°, $[\alpha]_D^{25}$ 0° (*c*, 0.2 in methanol); IR (KBr): ν_{max} 3510 (m), 3326 (m, OH), 1640 (m, C=N), 1613 (m), and 933 (w, OCH_2O) cm^{-1}; UV (methanol): λ_{max} (log ϵ) 241 (4.31), 263 (4.28), 291 (4.66), 310 (sh, 4.40), 341 (4.23), and 380 (sh, 3.83) nm; UV (methanol–0.1 *N* NaOH): λ_{max} (log ϵ) 252.5 (4.45), 311 (4.32), and 380 (4.44) nm; NMR (CD_3OD): 3.17, 4.74 (2 t, J = 6 Hz, CH_2CH_2), 4.02 (s, OCH_3), 6.33 (s, OCH_2O), 7.51 (s, 2H, aromatic), and 6.87, 7.64, 8.59, and 9.25 (4 s, 1H each, aromatic); electron-impact mass spectrum: *m/e* (%) 337 ($M^+ + O - H$, 6.2), 336 (8.6), 335 (10.2), 334 (8.5), 323 (19.4), 322 (M^+, 31.4), 321 (71.4), 320 (97.5), 319 (69.9), 318 (91.0), 307 (10.5), 306 (19.3), 305 (10.1), 278 (7.2), 277 (6.4), 261 (6.3), 260 (5.5), 202 (6.0), 177 (1.8), 176 (2.7), 160 (7.0), 159 (7.7), 130 (9.4), 52 (32.4), and 50 (100); chemical-ionization mass spectrum (isobutane as reagent gas):

[2] Melting points were taken with a Thomas-Hoover apparatus and are uncorrected. UV spectra were determined with a Hitachi model 200-20 spectrophotometer. IR spectra were taken with a Perkin-Elmer model 577 spectrophotometer in potassium bromide pellets. NMR spectra were recorded in suitable solvents, with tetramethylsilane as the internal standard, on a Varian A-60A or Bruker HX-90E spectrometer; chemical shifts are reported as δ (parts per million) values.

Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Mass spectra were obtained using a DuPont 21-491 double-focusing spectrometer *via* a direct-inlet probe, and the chemical-ionization mass spectra employed isobutane as the reactant gas. High-resolution mass spectra were determined on an A.E.I. MS-902 instrument using a direct-inlet system. Elemental analyses were performed by the Laboratory of Microanalysis, Chung-Shan Institute of Science and Technology, Chung-Li, Taiwan.

[3] A specimen of this plant was authenticated by Mr. Muh-Tsuen Kao, Department of Botany, National Taiwan University, and a voucher specimen was deposited in the School of Pharmacy of that University.

[4] IRA-410, chloride form.

[5] E. Merck No. 7734.

m/e (%): 378 (M^+ + 56, 4.0), 376 (2.9), 364 (M^+ + 42, 10.5), 362 (14.9), 337 (M^+ + O − H, 11.8), 336 (16.7), 335 (8.5), 324 (12.7), 323 (40.3), 322 (M^+, 93.8), 321 (90.6), 320 (100), 319 (65.9), 318 (16.3), 178 (1.8), 177 (1.8), 176 (2.7), and 148 (4.0). IR, UV, NMR, and mass spectral comparisons showed that this compound was identical to a synthetic sample of 3-hydroxy-2-methoxy-10,11-methylenedioxyberbinium chloride (II*a*), and a mixed melting-point determination showed no depression.

Reduction of II*a* to Q-4-H (IV*a*)—A solution of Q-4 (61 mg) in methanol (18 ml) was cooled on an ice bath and treated portionwise with sodium borohydride (122 mg). The mixture was stirred at room temperature for 24 hr and then evaporated under reduced pressure to remove methanol. The residue was treated with 6% NH_4OH (30 ml) and extracted with chloroform (4 × 30 ml). The combined chloroform extracts were washed with water (50 ml), dried (anhydrous potassium carbonate), and evaporated to give a syrup.

Crystallization from chloroform–methanol provided Q-4-H (30 mg) as pale-yellow plates, mp 142–144°; IR (KBr): ν_{max} 3571 (m, OH), 2930 (w), 2894 (w), 2853 (w), 2813 (w, *trans*-quinolizidine bands), 1505 (s), 1477 (s), and 930 (m, OCH_2O) cm^{-1}; UV (methanol): λ_{max} (log ϵ) 230 (sh, 4.00) and 290 (3.94) nm; UV (methanol–0.1 *N* NaOH): λ_{max} (log ϵ) 240 (sh, 4.01) and 296 (4.04) nm; NMR (CD_3OD): δ 2.49–3.97 (m, 9H, aliphatic), 3.85 (s, OCH_3), 5.86 (s, OCH_2O), 6.56 (s, 2H, aromatic), and 6.65 and 6.82 (2 s, 1H each, aromatic); electron-impact mass spectrum: *m/e* (%) 326 (13.6), 325 (M^+, 62.5), 324 (24.9), 310 (4.4), 178 (1.2), 177 (5.8), 176 (29.2), 150 (14.7), 149 (27.4), 148 (100), 147 (21.8), and 146 (4.6). The high-resolution mass measurement of *m/e* 325 (M^+) showed a value of 325.1315 for $C_{19}H_{19}NO_4$ (calc. 325.1312). The IR, UV, NMR, and mass spectra and a mixed melting-point determination showed that this compound was identical to an authentic sample of 3-hydroxy-2-methoxy-10,11-methylenedioxyberbine (IV*a*).

Synthesis of IV*a*—A mixture of 6-hydroxy-7-methoxy-1-(3′,4′-methylenedioxybenzyl)-1,2,3,4-tetrahydroisoquinoline (V*a*) (125 mg as the hydrochloride salt) and formaldehyde (4 ml) in acetic acid (4 ml) was refluxed for 3 hr. The reaction mixture was evaporated, and the resulting residue was made alkaline with 10% NH_4OH (30 ml). The aqueous solution was extracted with chloroform (4 × 30 ml), and the combined extracts were washed with water (50 ml), dried (anhydrous sodium sulfate), and evaporated to leave a residue (110 mg). The residue was purified further on a silica gel (7 g) column with the eluent (acetone in benzene) increasing from 1 to 20%.

Each 30-ml eluate was collected and analyzed by TLC. The residue from column fractions 18–61 was crystallized from chloroform–methanol to yield IV*a* (40 mg), mp 145–146°. The spectral properties of this compound were identical in every respect to those of an authentic sample synthesized by another route (8), mixed mp 144–145°. It also was identical to Q-4-H in terms of the IR, UV, NMR, and mass spectra and the mixed melting point (144–145°).

Anal.—Calc. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.02; H, 5.95; N, 4.22.

Synthesis of II*a*—A solution of IV*a* (30 mg) in 25% acetic acid (30 ml) was treated with a saturated solution of mercuric acetate in acetic acid (1.5 ml), and the resulting mixture was refluxed on a water bath for 1 hr. The reaction mixture was evaporated, and the residue was dissolved in anhydrous ethanol (30 ml). A stream of hydrogen sulfide gas was introduced into the solution to precipitate the mercuric ion as the sulfide salt. The precipitate was removed by centrifugation and filtration over a layer of filter aid. The residue was washed with ethanol (3 × 40 ml) and filtered.

The combined ethanolic filtrates were evaporated to leave a residue. This residue was dissolved in warm concentrated hydrochloric acid (10 drops) and set aside to crystallize. The crude crystals were collected and recrystallized from methanol to yield fine yellow needles (15 mg) of II*a*, mp 259–261°. Compound II*a* was identical to Q-4 in terms of their IR, UV, NMR, and mass spectra. The mixed melting point was 258–260°.

Anal.—Calc. for $C_{19}H_{16}ClNO_4 \cdot H_2O$: C, 60.73; H, 4.83; Cl, 9.43; N, 3.73. Found: C, 60.15; H, 4.67; Cl, 9.45; N, 3.84.

Isolation and Identification of Dehydrodiscretine (II*b*, Q-3)—The residue from column fractions 46–49, eluted with 30% methanol in chloroform, was combined (125 mg) and rechromatographed on a small silica gel (15 g) column. Elution was performed by using methanol in chloroform (increasing from 5 to 20%) and collecting each 15-ml fraction. Additional Q-4 (15 mg) was isolated from fractions 34–43. The residue from fractions 46–53 was dissolved in a minimum amount of methanol and set aside in a refrigerator for 3 days.

The fine reddish-brown needles of Q-3 (10 mg) were filtered, mp 230–234°, $[\alpha]_D^{25}$ 0° (*c*, 0.15 in methanol); IR (KBr): ν_{max} 3400 (m, OH), 1633 (m), 1614 (m), 1517 (s), 1500 (s), 1430 (s), 1358 (s), 1290 (s), 1220 (s), 1128 (m), 1008 (m), and 880 (m) cm^{-1}; UV (methanol): λ_{max} (log ϵ) 243 (4.24), 264.5 (4.21), 289 (4.48), 310 (sh, 4.36), 341 (4.19), and 379 (sh, 3.82) nm; UV (methanol–0.1 *N* NaOH): λ_{max} (log ϵ) 254 (4.42), 310 (4.24), and 378 (4.37) nm; NMR (CD_3OD): δ 3.89, 4.01, and 4.07 (3 s, OCH_3 each), 3.05 (t, J = 6 Hz, CH_2), 7.43 (s, 2H, aromatic), and 6.53, 7.33, 8.27, and 9.07 (4 s, 1H each, aromatic); chemical-ionization mass spectrum (isobutane as reagent gas): *m/e* (%) 353 (M^+ + O − H, 2.0), 339 (0.3), 338 (M^+, 0.3), 208 (1.0), 206 (0.7), 178 (1.7), 177 (1.1), 169 (3.0), 167 (7.5), 165 (1.2), 164 (3.0), 163 (1.4), 152 (3.6), 150 (14.5), 149 (14.3), 141 (14.8), 137 (8.3), 128 (5.5), 127 (100), 126 (19.9), 105 (8.2), 103 (3.2), 95 (4.2), 91 (3.2), 85 (5.3), 76 (6.6), 75 (34.6), 74 (87.8), and 73 (5.1). This compound was shown to be 3-hydroxy-2,10,11-trimethoxyberbinium chloride (II*b*) by direct comparison with a synthetic sample.

Reduction of II*b* to Discretine (IV*b*)—A solution of Q-3 (8 mg) in methanol (5 ml) was reduced with sodium borohydride (20 mg) following a procedure similar to that described for the reduction of Q-4. The reduced product was crystallized from methanol as colorless needles (3 mg) and was designated as Q-3-H. Additional Q-3-H was obtained from reduction of the mother liquid of Q-3, which also was admixed with Q-4. The reduced mixture of Q-3-H and Q-4-H was separated on a preparative TLC plate of silica gel[5] (1 mm) and eluted with acetone–benzene (1:3). The zone corresponding to Q-3-H (R_f 0.31) was scraped and extracted with methanol–chloroform (1:9). The extract was evaporated, and the residue was crystallized from methanol to provide 9 mg of Q-3-H.

The combined material of Q-3-H was recrystallized from methanol, mp 180–182°; IR (KBr): ν_{max} 3570 (m), 3440 (m, OH), 2940 (m), 2840 (w), 1615 (m), 1520 (s), 1448 (m), 1350 (m), 1260 (s), 1140 (m), and 1030 (m) cm^{-1}; UV (methanol): λ_{max} (log ϵ) 225 (sh, 4.18) and 286.5 (3.87) nm; UV (methanol–0.1 *N* NaOH): λ_{max} (log ϵ) 245 (4.08), 290.5 (3.96), and 300 (sh, 3.83) nm; NMR ($CDCl_3$): δ 2.50–3.80 (m, 9H, aliphatic), 3.86 (s, 2 OCH_3), 3.89 (s, OCH_3), 6.66 (s, 2H, aromatic), and 6.58 and 6.72 (2s, 1H each, aromatic); electron-impact mass spectrum: *m/e* (%) 342 (1.9), 341 (M^+, 7.8), 340 (3.4), 339 (3.0), 338 (4.3), 283 (5.3), 181 (3.5), 180 (2.4), 178 (2.8), 177 (3.6), 176 (12.0), 166 (11.9), 165 (38.1), 164 (100), 151 (3.0), 150 (4.7), 135 (4.1), and 133 (4.1). The high-resolution mass measurement of *m/e* 341 (M^+) showed a mass for $C_{20}H_{23}NO_4$ of 341.1624 (calc. 341.1626). This compound was identical to a synthetic sample of IV*b* in terms of their IR, UV, NMR, and mass spectra and a mixed melting-point determination.

Synthesis of II*b* from IV*b*—(±)-Discretine was synthesized according to the method of Kametani *et al.* (10, 11) by condensation of 6-hydroxy-7-methoxy-1-(3′,4′-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (V*b*) with formaldehyde or by debenzylation of *O*-benzyldiscretine. The pure product of IV*b* was crystallized from acetone–ether as white plates, mp 183–184° [lit. (10) mp 182–184°]. Direct comparison of their IR, UV, NMR, and mass spectra showed that this compound was identical to Q-3-H; a mixed melting point of 182–184° was observed.

A solution of IV*b* (300 mg) in acetic acid (12 ml) was added to a warm solution of mercuric acetate (2.1 g) in acetic acid (14 ml). The resulting mixture was refluxed on a water bath for 7 hr. Following a workup similar to that described for the synthesis of II*a*, a yellow residue was obtained. This residue was dissolved in concentrated hydrochloric acid (5 ml). The crude crystal was collected and recrystallized from methanol to yield II*b* as fine reddish-yellow needles (284 mg), mp 232–234°. This compound was identical to Q-3 in terms of their IR, NMR, and UV spectra, and a mixed melting point of 230–233° was obtained.

Anal.—Calc. for $C_{20}H_{20}ClNO_4 \cdot H_2O$: C, 61.30; H, 5.66; Cl, 9.05; N, 3.57. Found: C, 60.95; H, 5.68; Cl, 8.84; N, 3.64.

Isolation and Identification of Magnoflorine (III) Iodide (Q-6)—The crude brown residue (1.76 g) from column fractions 65–118, eluted with 75% methanol in chloroform, was dissolved in methanol. To this solution was added an excess of saturated methanolic potassium iodide solution. The solution was kept at room temperature for 2 days, and the resulting white precipitate was removed by filtration. The precipitate was crystallized twice from methanol to yield Q-6 (85 mg) as white crystals, mp 250–252°. This compound was identified as magnoflorine (III) iodide on the basis of its TLC behavior and IR, UV, circular dichroism, NMR, and mass spectral data, which were identical to those obtained for an authentic sample of magnoflorine iodide. In addition, there was no depression in the mixed melting-point determination with an authentic sample of magnoflorine iodide.

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Acetylcarnitine and Cholinergic Receptors

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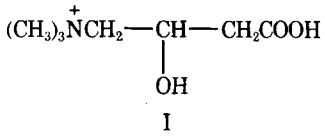
Abstract □ Acetylcarnitine, a naturally occurring compound found in high concentration in heart and skeletal muscle of vertebrates, bears structural resemblance to acetylcholine, and studies have shown that it has slight cholinergic properties. Acetylcarnitine was subjected to conformational analysis by extended Hückel theory (EHT) and complete neglect of differential overlap (CNDO/2) molecular orbital methods. The preferred conformations were examined with respect to their similarity to the Kier and Chothia–Pauling models of cholinergic receptor patterns. The preferred conformations of both isomers did not fit the receptor pattern described by Kier's model, although energy barriers to rotation are low enough to permit accommodation. The Chothia–Pauling model predicts activity for the *S*-isomer only. These studies partially explain the low cholinergic activity found for acetylcarnitine and the higher activity of (*S*)-acetylcarnitine compared to the *R*-isomer.

Keyphrases □ Acetylcarnitine—conformational analysis, comparison to cholinergic receptor patterns □ Cholinergic activity—acetylcarnitine, conformational analysis, comparison to cholinergic receptor patterns □ Structure–activity relationships—acetylcarnitine, conformational analysis, comparison to cholinergic receptor patterns

L-(−)-Carnitine [(*R*)-carnitine, I] is a naturally occurring substance found in high concentrations in plants, microorganisms, and animals. The muscles of vertebrates and invertebrates have especially high levels. Its principal physiological function is to transport free fatty acids into the mitochondria prior to their oxidation *via* the β-oxidation cycle (1).

(*R*)-Acetylcarnitine (II), a derivative of carnitine, also is found in high concentrations in the tissues of vertebrates. Enveloped within the structure of acetylcarnitine is the structure of the parasympathetic neurotransmitter acetylcholine (indicated by the dashed lines in II). The close structural similarity of acetylcarnitine to acetylcholine has



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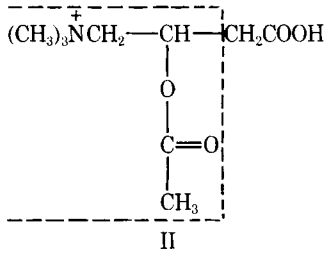

II

prompted investigations into its pharmacological properties as either a cholinomimetic or an anticholinergic compound (2–11).

BACKGROUND

It was reported that high doses of acetylcarnitine exerted a cholinergic effect on the heart which was blocked by atropine (2). Strack and Försterling reported that carnitine had little biological activity (3). However, Dallemagne *et al.* (4) found that carnitine and acetylcarnitine depolarized motor end plates. They reported that (*R,S*)-carnitine was 1/1000 as potent as acetylcholine on frog rectus abdominis muscle and that (*R*)-acetylcarnitine was 10–25% as potent as acetylcholine (4).

In contrast, Blum *et al.* (5) found (*R*)-carnitine to have 1/10,000 and (*S*)-carnitine to have 1/4000 the activity of acetylcholine on rectus abdominis muscle. They also reported that (*R*)- and (*S*)-acetylcarnitine were 1/700 as potent as acetylcholine in lowering arterial blood pressure in dogs (5). They concluded that acetylcarnitine acts at the cholinergic site rather than by blocking acetylcholinesterase. Two other groups found that synthetic (*R,S*)-acetylcarnitine possessed only weak to no nicotinic activity (6, 7).

Hosein and Proulx (8) claimed to have isolated a cholinergic active fraction of brain synaptosomes and identified this fraction as containing the coenzyme A ester derivatives of γ-butyrobetaine, crotonbetaine, carnitine, and acetylcarnitine. Six groups of investigators were unable to verify Hosein's isolation and identification of the active cholinergic substance of the brain, and the substance isolated by Hosein *et al.* apparently was acetylcholine itself (7). Falchetto *et al.* (9) reported that cortical neurons excited by acetylcholine also are excited by carnitine and acetylcarnitine. Carnitine was found to be more potent than acetylcarnitine.

Fritz (10) injected (*R,S*)-acetylcarnitine intracisternally into rat brains and evoked pronounced motor activity, which was not observed with

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