73–74° (F mél avec échantillon auth.[3] non abaissé). i.r.: 1740 (CO), 1520–1342 (NO^2) cm^{-1}. RMN (voir texte) et δ = 3,98 (s.3H — OCH_3), 4,05 (s.6H — OCH_3) ppm.

Acide dihydroxy-2,5 nitro-3 benzoïque (ou acide nitro-3 gentisique) (VI). 20 mg de (I) sont déméthylés dans cm^3 de HBr à 48% pendant 1 hr à ébullition. On ajoute 10 cm^3 d'eau glacée, on extrait à l'éther. L'éther est ensuite extrait par une solution de bicarbonate de Na à 5 %. Après acidification, on isole 10 mg de (VI). Recristallisé dans l'eau, F: 226–228° (P.F. non abaissé en mélange avec échantillon préparé par synthèse selon Klemenc[3]). I.r. superposable (KBr): 3500 (OH), 1680 (CO), 1520–1342 (NO_2) cm^{-1}. Spectre masse: *m/e* (%) M^+ 199 (25), 181 (100), 165 (5), 135 (10), 123 (5), 107 (8), 95 (15), 79 (20), 53 (25).

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RUTACEAE

A QUATERNARY TETRAHYDROPROTOBERBERINE ALKALOID FROM *FAGARA CAPENSIS*

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Abstract—The quaternary tetrahydroprotoberberine alkaloid, (—)-*N*-methyltetrahydropalmatine, has been isolated from the stem bark of *Fagara capensis* Thunb. This is the first report of the isolation of this alkaloid from a natural source

INTRODUCTION

IN AN earlier communication[1] the presence of the furoquinoline alkaloid, skimmianine and of the benzophenanthridine alkaloids, chelerythrine and nitidine, was reported in the stem and root barks of *Fagara capensis* Thunb. (Rutaceae). We now report the isolation and characterization of a quaternary tetrahydroprotoberberine alkaloid, (—)-*N*-methyltetrahydropalmatine, (I), from the stem bark of this species. Previously, (—)-α-canadine methochloride was the only tetrahydroprotoberberine alkaloid reported in the genus *Fagara*[2–5] and the closely allied genus *Zanthoxylum*.[6] The tertiary bases, (+)-tetrahydropalmatine and (±) tetrahydropalmatine, have been reported in the genus *Corydalis* (Papaveraceae) and (—)-tetrahydropalmatine from *Stephania* (Menispermaceae),[7] but this is the first isolation of (—)-*N*-methyltetrahydropalmatine from a natural source.

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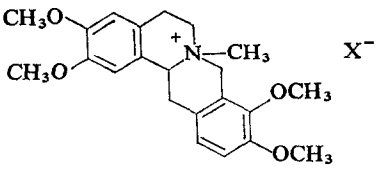
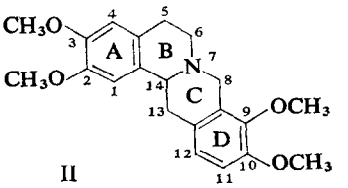
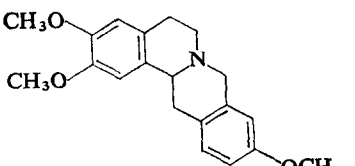


I (a) $X = NO_3^-$
(b) $X = I^-$



II



III

RESULTS AND DISCUSSION

The u.v. spectrum of the colourless needles, from the stem bark, was indicative of a tetrahydroprotoberberine.[8] The compound gave a negative Labat test[9] and the i.r. spectrum confirmed absence of methylenedioxy groups. Chemical tests and the absence of i.r. absorption above 3050 cm^{-1}, other than that due to free water (3400 cm^{-1}), indicated the lack of phenolic groups. In the i.r. spectrum, the presence of strong bands at 1240 cm^{-1} and 1280 cm^{-1} indicated an AR-O- group (typically methoxyl).

The alkaloidal nitrate was hygroscopic and on recrystallisation from ethanol, entrained solvent. This problem was overcome by conversion of the nitrate to the sparingly water-soluble iodide which on distillation *in vacuo* (260–280°/0·08 mm Hg) gave a tertiary base,

TABLE 1. PROTON MAGNETIC RESONANCE DATA ON TERTIARY AND QUATERNARY TETRAHYDROPROTOBERBERINE BASES

	Compound	Solvent	Aromatics	—O—CH_3	—$\overset{+}{N}$—CH_3	C-8 Protons	
Ia	(—)-*N*-Methyltetrahydropalmatine nitrate	D_2O	2·85–2·91	6·02–6·10	6·59	5·10	broad apparent singlet
Ib	(—)-*N*-Methyltetrahydropalmatine iodide	DMSO-D_6	2·85–3·00	6·12–6·18	6·65	5·05	broad apparent singlet
II	Tetrahydropalmatine	$CDCl_3$	3·12–3·35	6·17	—	5·70	A part of AB quartet $J = 16$ Hz
III	Norcoralydine[10]	$CDCl_3$	2·90–3·20	5·95	—	5·70	broad apparent singlet

(T.M.S. = τ10).

[8] A. W. SANGSTER and K. L. STUART, *Chem. Rev.* **65**, 69 (1965).
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$C_{21}H_{25}NO_4$, as almost colourless needles, m.p. 138–139°. The u.v. spectrum of this tertiary base differed only marginally from those of the quaternary salts and was identical with that of an authentic sample of tetrahydropalmatine (II). Elemental analysis of the quaternary iodide ($C_{22}H_{28}INO_4$) suggested an *N*-methyl derivative of tetrahydropalmatine.

A PMR study (Table 1) of both the (—)-*N*-methyltetrahydropalmatine nitrate (Ia) in D_2O and the iodide (Ib) in DMSO — D_6, indicated the presence of four aromatic methoxyl groups at about τ 6 and a broad quaternary *N*-methyl signal at τ 6·59 (nitrate) and τ 6·65 (iodide). The tertiary base (II) exhibited the lowfield doublet of the C-8 quartet centred on τ 5·70, $J = 16$Hz. The highfield portion of the AB pattern was obscured by the methoxyl signals at τ 6·17. These data were compatible with the alkaloid, Ia, possessing 9,10-dimethoxy substituents.[10,11]

Previous investigators have established the major fragmentation pathways of tetrahydroprotoberberine alkaloids on electron impact.[10–12] Chen and MacLean[10] demonstrated that the intensity of the *m/e* 149 peak was far greater in those alkaloids bearing 9,10 dimethoxy substituents than in those with 10,11 dimethoxy groups. The tertiary base, tetrahydropalmatine (II) exhibited a 49 per cent relative abundance of *m/e* 149 ion in contrast to the 14 per cent seen in norcoralydine (III). (—)-*N*-Methyltetrahydropalmatine iodide (Ib) and nitrate (Ia) each exhibited a mass spectrum almost identical with that of the tertiary base (II). The nitrate, however, exhibited an intense *m/e* 369 ($P^+ - 1$) ion indicative of its more facile Hofmann elimination at the same ion-source temperature.

The alkaloid isolated from *Fagara capensis* Thunb. has been shown unequivocally to be (—)-*N*-methyltetrahydropalmatine. The stereochemistry of the B/C ring junction has not been established but work is proceeding to that end.

EXPERIMENTAL

Plant Material

Stem bark of *Fagara capensis* Thumb. collected in the eastern Cape Province, South Africa. The material was authenticated by the Forest Research Institute, Pretoria, South Africa.

Extraction

The powdered bark (3 kg) was successively extracted in a Soxhlet with light petroleum (b.p. 40–60°), $CHCl_3$, and methanol to exhaustion. Each extract was concentrated under reduced pressure at 60°. The concentrated $CHCl_3$ extract (300 ml) was extracted with 0·1 N HCl (3 × 50 ml and 4 × 30 ml) and the acidic fractions collected. A yellowish precipitate formed at the interface and was reserved. The bulked acid fraction was extracted with $CHCl_3$ (ethanol-free) (3 × 150 ml) in a liquid–liquid extractor. The dried extract was dissolved in dry ethanol (40 ml) and 6N HNO_3 (0·5 ml) added. The resultant yellow precipitate of chelerythrine nitrate[1] was removed and the filtrate refrigerated overnight. Pale yellow needles formed and after several recrystallisations from dry ethanol yielded colourless needles (645 mg) m p. 169–170°.

(—)-N-*Methyltetrahydropalmatine Nitrate* (*Ia*)

Isolated as colourless needles m.p. 169–170°, $[\alpha]_D^{24}$ — 263·6° (C. 0·222 water); λ_{max}^{EtOH} 214, 232·5, 284 nm (log ϵ 4·57, 4·20, 3·73) λ_{min}^{EtOH} 257 nm (log ϵ 2·91); i.r. ν cm^{-1}) 1240, 1280, ($-OCH_3$) 1350 (NO_3^-); when examined on cellulose chromatoplates (wet thickness 250μ) using 0·1 N HCl as solvent, a single spot (R_f 0·83) was obtained.

[11] A. D. CROSS, L. DOLEJS, V. HANUS, I. MANN and S. PFEIFER, *Tetrahedron Letters* 83 (1967).
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(—)-N-*Methyltetrahydropalmatine Iodide* (*Ib*).

(—)-*N*-Methyltetrahydropalmatine nitrate (130 mg) was dissolved in water (0·6 ml) and saturated KI solution (five drops) added. The resultant heavy white precipitate on recrystallisation from dry methanol yielded minute colourless needles (107 mg) m.p. 203–204°; $[\alpha]_D^{24}$ — 258·2° (C. 0·22 methanol); λ_{max}^{EtOH} 214, 232 (sh.), 284 nm (log ϵ 4·62, 4·34, 3·75) λ_{min}^{EtOH} 257 nm (log ϵ 2·98); Elemental analysis: Found: C, 53·13; H, 5·79. Calc. for $C_{22}H_{28}INO_4$: C, 53·10; H, 5·67%; when examined on cellulose chromatoplates (wet thickness 250μ) in 0·1 N HCl, a single spot (R_f 0·83) was obtained.

N-*Demethylation of* (—)-N-*Methyltetrahydropalmatine Iodide*

(—)-*N*-Methyltetrahydropalmatine iodide (20 mg) was vacuum distilled (260–280°/0·08 mm Hg) to yield a yellowish glass which on trituration with methanol yielded pale yellow needles (8 mg) m.p. 138–139°. The i.r. spectrum was identical with that of an authentic sample of tetrahydropalmatine (II) and gave identical R_fs on alumina and silica gel plates.

The u.v. spectra were recorded in ethanol and the i.r. spectra in KCl. PMR (60 MHz) spectra were recorded in the solvent indicated (Table 1) with T.M.S. as internal standard (τ10). Mass spectra were determined on an A.E.I. MS902 double-focussing mass spectrometer with the compound introduced *via* the direct insert probe. M.ps. (Kofler hot stage) are uncorrected. The optical rotations were measured in an 0·5 dm tube on a Pepol '60' spectropolarimeter. TLC plates were developed at 25°.

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SOLANACEAE

5,20α(R)-DIHYDROXY-6α,7α-EPOXY-1-OXO-(5α) WITHA-2,24-DIENOLIDE, A NEW STEROIDAL LACTONE FROM *WITHANIA COAGULANS*

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Abstract—The structure of the title compound (I) has been elucidated by analysis of the spectral data, as well as by its fragmentation pattern under electron impact. The configuration at C-20 (R) has been determined by NMR, and the one at C-22 (R) is based on CD measurements.

THE WITHANOLIDES are a series of C_{28} steroidal lactones with various substitution patterns, which have been isolated from different species of the Solanaceae family: *Withania som-*