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THE ^{13}C-NUCLEAR MAGNETIC RESONANCE SPECTRA OF PHYSOSTIGMINE AND RELATED COMPOUNDS*

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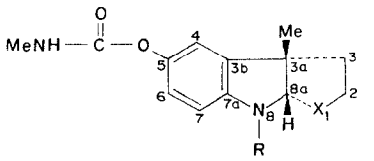
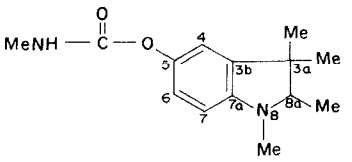
Key Word Index—*Physostigma venenosum*; Leguminosae; Calabar bean; seeds; alkaloids; ^{13}C-NMR spectra.

The medicinally valuable alkaloid, physostigmine (**1a**), has been long established as the major basic component of the seeds of *Physostigma venenosum* (Calabar beans) [1]. More recently the alkaloids physovenine (**1b**), eseramine (**1c**) and N_8-norphysostigmine (**1d**) have also been extracted as minor basic components of these seeds [2,3].

As well as containing ring systems which are likely to be biogenetically derived from tryptophan, these alkaloids all possess *N*-methylcarbamyl groups. Although the *O*-carbamyl group is present in the structure of novobiocin [4], and the *N*-carbamyl group is present in the biogenetic intermediates citrulline [5] and carbamyl aspartate [6], its presence in the bases of *Physostigma venenosum* seeds, where it is probably biogenetically derived from carbamyl phosphate (cf. refs. [5] and [6]) is as yet unique in higher plant alkaloids. As a prerequisite to a biosynthetic study on this group of alkaloids and as a useful aid in the structural investigation of further bases isolated from this source, we have examined the ^{13}C-NMR spectra of the above four alkaloids and report below (Table 1) their full assignment.



(**1a**) R = Me; X = NMe
(**1b**) R = Me; X = O
(**1c**) R = Me; X = N·C·NHMe
(**1d**) R = H, X = NMe



(**2**)

The carbon assignments are based on proton noise decoupled, off-resonance decoupled and proton coupled spectra together with comparison with literature data [7] and the ^{13}C-NMR spectrum of the analogous indoline (**2**) [8]. The correlations are generally very close, but useful diagnostic shifts are seen in the series, particularly at C-2 where a downfield shift of 14.1 ppm for physovenine (**1b**) and an upfield shift of 7.5 ppm for eseramine (**1c**) are observed, relative to the C-2 value in physostigmine. Similarly C-8a exhibits an upfield shift of 7.8 and 8.9 ppm in N_8-norphysostigmine (**1d**) and eseramine (**1c**) respectively, and a downfield shift of 6.6 ppm in physovenine (**1b**). The 8-methyl group also shows an upfield shift of 7.2 and 4.8 ppm respectively in physovenine (**1b**) and eseramine (**1c**).

EXPERIMENTAL

The ^{13}C-NMR spectra were recorded at 20 MHz on a Varian CFT-20 spectrometer in the quoted solvent. Chemical shifts are given in ppm relative to TMS as internal standard. The concentrations of the solutions used were 10–20%, and the spectra were recorded at approximately 30°.

Acknowledgements—We thank the SRC for financial assistance in the purchase of a Varian CFT-20 spectrometer.

* Part 11 in the series "Alkaloids of *Physostigma venenosum*". For Part 10 see Dale F. J. and Robinson B. (1970) *J. Pharm. Pharmacol.* **22**, 889.

Table 1. ^{13}C-NMR spectral assignments in *Physostigma venenosum* alkaloids

Compound (solvent)	Assignment (ppm) 1	2	3	3a	3a-Me	3b	4*	5	6*	7	7a	8-CH_3	8a	CH_3NH†	CO†
Physostigmine (**1a**) ($CHCl_3$)‡	36.9	53.2	40.7	52.6	27.2	137.4	116.1	149.3	120.4	106.5	143.3	38.4	98.1	27.5	156.3
N_8-Norphysostigmine (**1d**) ($CHCl_3$)	37.0	52.5	40.7	53.7	26.9	137.8	116.5	146.9	120.5	109.0	144.0		90.3	27.9	156.3
Eseramine (**1c**) (DMSO)	CO—157.7 Me—23.3	45.7	38.5	50.4	26.9	135.1	116.0	147.4	120.7	105.8	142.8	33.6	89.2	26.9	155.6
Physovenine (**1b**) ($CHCl_3$)	—	67.3	41.6	52.3	24.6	135.2	116.5	147.9	120.8	105.5	143.0	31.2	104.7	27.7	156.3
Compound (**2**) ($CHCl_3$)	—	—	—	42.7	12.0 22.7	140.0	115.5	149.2	120.0	107.4	143.8	34.1	72.9 Me 25.5	27.4	156.3

* These assignments are interchangeable; † compare $MeNHCOOC_2H_5$ (Me—27.4; CO—157.8 ppm) [7]; ‡ coupling constants: J_1 (CH-7)—160.1; J_1 (CH-6/4)—160.8 and 158.8; J_1 (CH-3a-Me)—127; J_1 (CH-8-Me)—134; J_1 (CH-8a)—155; J_1 (CH-2)—142; J_1 (CH-3)—125; J_1 (CH-MeNH)—138; J_3 (CH-4/6)—3.9 and 4.9 Hz.

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ALKALOIDS OF THREE *ASPIDOSPERMA* SPECIES

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Key Word Index—*Aspidosperma formosanum, A. campus-belus, A. desmanthum*; Apocynaceae; isolation; olivacine; uleine; 3-epiuleine; 1,13-dihydro-13-hydroxyuleine; aspidocarpine; lichexanthone; phthalimide; aspidoalbine.

Plants and sources. Aspidosperma formosanum A. P. Duarte (Formosa, Goiás, Brazil, 1965; APD herbarium register 9387); *A. campus-belus* A. P. Duarte (Campos Belos, Goiás, 1965, APD register 9481); *A. desmanthum* Benth. ex Müll.-Arg. (IPEAN, Belém, Pará, Brazil, 1965, APD register 9798). *Previous work*: None; *A. formosanum* is systematically close to *A. dasycarpon* [1] (Series Tomentosa); *A. campusnbelus* to *A. nigricans* [2] (Series Pyricolla); *A. desmanthum* to *A. exalatum* [3], *A. spruceanum* [2a], and *A. album* [4] (Series Nobile).

Bark. Hot continuous EtOH extraction followed by concn gave in each case about 10% syrupy extract. This was macerated with 2N HOAc, filtered, and divided into standard fractions [5] (letter code; method of obtention; percent of extract in the case of *A. formosanum, A. campus-belus*, and *A. desmanthum*, respectively); A, C_6H_6 extraction of the aq. HOAc solution, 0.87, 2.1, 1.47; B, $CHCl_3$ extraction of the same, 8.7, 1.4, 4.47; C, $CHCl_3$ extraction of the solution after neutralization with HCO^-_3, 2.53, 7.1, 2.2; D, $CHCl_3$ extraction after basification to pH 13 with NaOH, 1.75, 0.8, 0.83.

In the preliminary testing of the various extracts, olivacine (**1**) was noted as the principal base in fraction B of *A. campus-belus*, and a small quantity obtained by direct crystallization from MeOH was compared satisfactorily with material from *A. nigricans* [2].

In large-scale work, the following compounds were isolated (plant; fraction(s), isolation methods, compound name and structure number, yield based on dried bark, mp, other relevant data for characterization, confirmation of identity):

A. desmanthum. C, direct crystallization from MeOH, aspidoalbine (**2**), 0.05%, 174–175° (lit. 174–177° [4], 168° [2a]); MS showing possible impurity of the *N*-acetyl analogue (**3**) at *m/e* 414, but not evident in the NMR; comparison of spectral data [4].

A. formosanum. (1) A,B,C; direct crystallization from MeOH, or basic Al_2O_3 III eluting with hexane–C_6H_6 (1:1) to C_6H_6, or with toluene to toluene–EtOAc (1:1), or with hexane–CH_2Cl_2 (4:1) to CH_2Cl_2, or Si gel eluting with EtOAc–MeOH (9:1); uleine (**4**); 0.64%; 72–78°, but highly variable (known to be poorly crystalline and solvated and show wide melting ranges [2,6]); $[\alpha]_D^{27}$ +20° ($CHCl_3$; *c* 0.94), λ_{max}^{MeOH} nm 213, 307, 315 (log ε 4.38, 4.28, 4.24), $\nu_{max}^{CHCl_3}$ cm^{-1} 3534*m*, 2941*s*, 1767*w*, 1637*m*, 1621*m*, 1460*s*, 1445*s*, 1314*s*, 1148*m*, 1125*m*, 1098*m*, 1047*m*, 1007*m*, 977*w*, 935*w*, 911*w*, 873*s*, 839*m*, NMR (100 MHz, $CDCl_3$) δ 8.72 (1Hs, eliminated with D_2O; NH), 7.40–6.80 (4Hm; ArH), 5.18 and 4.84 (2 × 1Hs; $=CH_2$), 3.95 (1Hd, *J* 3 Hz; C-4), 2.16 (3Hs; N-Me), 1.04 (2Hq, *J* 6 Hz; C-14), and 0.76 (3Ht, *J* 6 Hz; C-15), MS M^+ 266 (100%) and fragmentation as published [7], comparison with an authentic sample (B. Gilbert). *Significance*: the large amount of this alkaloid present, its relatively facile isolation, and its unusual and suggestive 1-methylene-4-aminotetrahydrocarbazole structure, have led us to explore chemical transformations into analogues of antischistosomicidal drugs (preazaquinone methides), which will be reported upon in another Journal.

(2) A,B, after preliminary crystallization of uleine; neutral Al_2O_3 I eluting with hexane–C_6H_6 (4:1); 3-epiuleine (**5**); 0.013%; amorphous; UV identical to that of uleine, $\nu_{max}^{CHCl_3}$ cm^{-1} 3521*m*, 2941*s*, 1767*w*, 1637*m*, 1621*m*, 1460*s*, 1445*s*, 1314*s*, 1140*m*, 1125*m*, 1101*m*, 1043*m*, 1010*m*, 978*m*, 952*w*, 910*w*, 870*s*, 823*m*, NMR MHz, $CDCl_3$) δ 7.98

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