11-METHOXY-MACUSINE A. A NEW QUATERNARY ALKALOID FROM *STRYCHNOS ANGOLENSIS*

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ABSTRACT.—The major quaternary alkaloid from *Strychnos angolensis* was isolated, and its structure was determined by its spectral data as 11-methoxy-macusine A. The alkaloid showed muscle-relaxant activity.

In our phytochemical and pharmacological screening of African *Strychnos* species six specimens of *Strychnos angolensis* showed convulsant and muscle-relaxant activity. One of the specimens (Lg 7844) was studied more in detail to find the alkaloids responsible for its pharmacological activity. The convulsant activity was found among the nonpolar alkaloids that were isolated, and their structure was elucidated (1).

In continuation of this study, we now want to report the isolation and structure of one of the alkaloids with muscle-relaxant effect.

RESULTS AND DISCUSSION

CHEMICAL STUDY.—The quaternary alkaloid fraction was separated by means of column chromatography, yielding the major alkaloid in pure, crystalline form. Field desorption ms of this alkaloid gave a molecular weight of 397. Electron impact ms showed a major fragment at *m/z* 382 (M^+-15), the demethylated alkaloid. The *m/z* 382 fragment is accompanied also by a rather abundant *m/z* 381 fragment, which points to a tetrahydro-β-carboline structure (2). Further important fragments were observed at *m/z* 198 and 199. Similar fragments have been reported for lochneram iodide (2-5). Non-aromatic substituted sarpagan derivatives have major fragments at *m/z* 168 and *m/z* 169. Therefore, an aromatic methoxy substituted sarpagan structure was concluded for the alkaloid. Fragments at *m/z* 323 (382-59) and 337 (396-59) points to the presence of a $COOCH_3$ group; fragments at *m/z* 351 (382-31) and 365 (396-31), to the presence of a CH_2OH group. These features fit into a macusine A or C type of alkaloid. The 1725 cm^{-1} absorption in the ir also points to the presence of a $COOCH_3$ group. In the ^{1}H-nmr, the deshielded quaternary *N*-methyl group is observed at 3.02 ppm. From the downfield shift of the carboxy-methyl group at 3.70 ppm, it is concluded that the carbomethoxy group is situated over the aromatic ring (6-10); thus, the C-16 configuration is similar to polyneuridine and macusine A (16 R).

The site of the aromatic methoxy group was determined from the ^{13}C-nmr and ^{1}H-nmr data. The ^{1}H-nmr showed a doublet with a large coupling constant and another with a small one, as well as a doublet of doublets in the aromatic region, which corresponds to the aromatic protons in a 10- or 11-methoxy substituted alkaloid. The shifts observed in the ^{13}C-nmr spectrum for the aromatic carbons are in accordance with those reported for 11-methoxy substituted indoles (11). The shift of 3.70 ppm of the aromatic methoxy group is similar to the shift reported for this group in gardnerine (11-methoxy) (6). The uv maxima are also similar to those reported for gardnerine (6).

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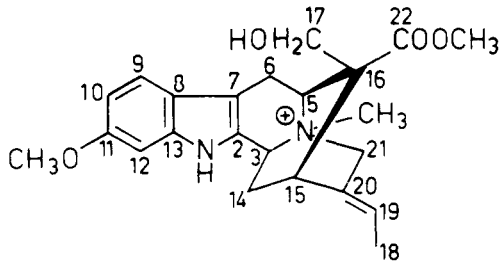

FIGURE 1. 11-Methoxy-macusine A.

Further evidence for the quaternary sarpagan-like structure was also obtained from the ^{13}C-nmr spectrum, in which the signals of C-5, C-3, and C-21 are shifted downfield considerably (*ca.* 11-14 ppm) if compared with a tertiary sarpagan-type alkaloid (12-14). The presence of two doublets and a triplet at about 60-70 ppm is indicative of the C-5-C-16 linkage. Thus, the structure of the alkaloid is 11-methoxy-macusine A.

The stereochemistry of the ethylidene side chain can be deduced from the shifts of C-15 in the ^{13}C-nmr (13). The shift of 28.6 ppm for C-15 is in accordance with a *cis* position of C-18 towards C-15, *i.e.* the normal geometry of this side chain.

The occurrence of the 11-methoxy-macusine A in *S. angolensis* is in accordance with the presence of a series of 11-methoxy substituted tertiary alkaloids in this plant (1), thus showing a preference for 11-methoxy substitution in its biosynthesis route of alkaloids.

PHARMACOLOGICAL STUDY.—Because the aqueous fraction showed considerable muscle-relaxant effect the isolated major alkaloid, 11-methoxy-macusine A was subject to some preliminary pharmacological tests. The results are summarized in table 1.

TABLE 1. Muscle-relaxant effect of the aqueous fraction of *S. angolensis* and 11-methoxy-macusine A

Material	Muscle-relaxant effect			Minimal lethal dose (mg/kg)
	Screen-grip in mice		Rat diaphragm 50% inhibition (mg/kg)	
	Graded response[a]	Dose (mg/kg)		
Aqueous fraction	X	50	0.27	75
11-methoxy-macusine A	X	75	0.16	100

[a]Mouse falls off the screen when it is inverted.

Indeed, the alkaloid showed muscle-relaxant activity in the screen-grip test, but at a higher dosage than did the aqueous fraction. 11-Methoxy-macusine A is also less toxic, which suggests that the aqueous fraction contains other active components.

The explanation of the results obtained might be that both 11-methoxy-macusine A and the alkaloids in the aqueous fraction have both peripheral and central effects. The alkaloids in the aqueous fraction show a more pronounced central effect (reflected by higher toxicity and lower dose for the screen-grip test), whereas the 11-methoxy-macusine A exerts a stronger peripheral action (showed by the diaphragm test).

Macusine A and C have not yet been the subject of pharmacological investigations. However, the related alkaloid macusine B, which lacks the carbomethoxy group at C-16, has shown convulsant activity (15) and was found to block α-adrenergic receptors, stimulate β-adrenergic receptors, and inhibit tryptamine receptors (16).

EXPERIMENTAL

PLANT MATERIAL.—The plant material, collection no. Lg 7844, was collected by Prof. F. Sandberg and Dr. A.J.M. Leeuwenberg in the period June-July 1970 in Kribi, Cameroon. The material was identified as *Strychnos angolensis* Gilg., by Dr. A.J.M. Leeuwenberg, and an herbarium specimen is kept at the Herbarium, Department of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen, The Netherlands.

EXTRACTION.—Ground root and stem bark (16 kg) were extracted twice with 65 liters of 1% acetic acid at room temperature. The aqueous extract was acidified with 5% HCl to pH=2. Mayer's reagent was then added until no more precipitate was formed. The precipitate was collected and dissolved in acetone-methanol-water (6:2:1) and filtered. The solution was run through a column packed with Amberlite IRA 400 in the Cl^- form. After evaporation of the acetone and methanol under reduced pressure, the aqueous solution of the alkaloid chlorides was basified with 10% ammonia and extracted with dichloromethane. Both the aqueous and dichloromethane fractions were taken to dryness.

ISOLATION OF 11-METHOXY-MACUSINE A.—Of the aqueous fraction (see above), 25 g was chromatographed on a silica gel column (Merck, Silica gel 60, 70-230 mesh) with the eluant methanol-0.2 M ammonium nitrate (3:2). The different fractions were tested on tlc (DC-Fertigplatten, Silica gel 60 F_{254}, 0.25 mm) with the solvent system methanol-0.2 M ammonium nitrate (3:2). The alkaloids were detected with iodoplatinate spray reagent. 11-Methoxy-macusine A was isolated in almost pure form from the column. Needle-like crystals of the alkaloid were obtained after recrystallization in chloroform.

CHARACTERIZATION OF 11-METHOXY-MACUSINE A.—The melting point of the chloride salt is 224-226°. The uv spectrum (in MeOH) showed maxima at λ 262, 266(sh), 293 and 300(sh) nm. The ir spectrum (KBr) showed major absorption bands at γ 3150, 2920, 1725, 1625, 1450, 1260, 1210, and 750 cm^{-1}. The ^{1}H-nmr spectrum (100 MHz, deuteropyridine) showed characteristic signals at 8.55 (1H, NH), 7.45 (1H, d, J=8 Hz, H-9), 7.32 (1H, d, J=2 Hz, H-12), 6.97 (1H, dd, J=2 Hz and J=8 Hz, H-10), 3.70 (6H s, 2 x OCH_3), 3.02 (3H, s, NCH_3) and 1.70 ppm (3H, d, H-18). The ^{13}C-nmr spectrum (25.2 MHz, CD_3OD) showed signals at 171.5 (s, C-22), 157.8 (s, C-11), 139.2 (s, C-13), 130.2 (s, C-2), 128.2 (s, C-20), 122.0 (d, C-19), 120.3 (s, C-8), 119.4 (d, C-9), 110.7 (d, C-10), 102.7 (s, C-7), 95.8 (d, C-12), 67.8 (d, C-5), 67.3* (t, C-21), 65.5* (t, C-17), 62.1 (d, C-3), 55.9 (q, OCH_3), 52.7 (s, C-16), 52.4 (q, OCH_3), 48.6 (d/q, NCH_3), 29.7 (t, C-14), 28.7 (d, C-15), 20.9 (t, C-6), and 13.0 ppm (q, C-18). (Signals marked with * may be interchanged.)

The ms showed characteristic fragments at (70 eV, 200°) *m/z*: 397(15), 396(46), 383(26), 382.1893(96, $C_{22}H_{26}N_2O_4$=382.1892), 381(59), 367(17), 366(22), 365(22), 353(22), 353(87), 351(100), 337(22), 336(15), 323(20), 322(20), 292(30), 279(48), 269(26), 268(48), 259(22), 205(17), 200(20), 199(67), 198(67), 197(17), 186(26), 184(24), 173(28), 174(17), 161(20), and 160(87). The fdms showed fragments at *m/z*: 397(100%), 383(12), 382(44), 367(20), and 351(18).

PHARMACOLOGY.—Screen-grip test was performed on female mice (18-20 g) of the NMRI strain by the method of Sandberg *et al.* (17). Both the aqueous fraction and the isolated alkaloid, 11-methoxy-macusine A, dissolved in saline were injected intraperitoneally. A rat diaphragm preparation (18) was used to further investigate the muscle-relaxant properties of the aqueous fraction and of the pure alkaloid. The final concentration in the organ bath, which produced 50% inhibition of the electrically stimulated muscle, is given. The doses were calculated by plotting the percentage; response in probits of the muscular contraction versus log dose.

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LITERATURE CITED

1. L. Bohlin, W. Rolfsen, J. Strömbom, and R. Verpoorte, *Planta Med.*, **35**, 19 (1979).
2. M. Hesse, "Progress in Mass Spectrometry," Vol. 1: Indolalkaloide, Verlag Chemie, Weinheim, 1974, p 156.
3. L.D. Antonaccio, N.A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J.M. Wilson, L.J. Durham, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 2161 (1962).
4. G.B. Marini-Bettòlo, S.A. Giuffra, C. Galeffi, and E.M. delle Monache, *Gazz. Chim. Ital.*, **103**, 591 (1973).
5. M. Ohashi, H. Budzikiewicz, J.M. Wilson, C. Djerassi, J. Lévy, J. Gosset, J. Le Men, and M.M. Janot, *Tetrahedron*, **19**, 2241 (1963).

6. S. Sakai, A. Kubo, T. Hamamoto, M. Wakabayashi, K. Takahashi, H. Ohtani, and J. Haginiwa, *Chem. Pharm. Bull.*, **21**, 1783 (1973).
7. G. Lewin, N. Kunesch, A. Cavé, T. Sevenet, and J. Poisson, *Phytochemistry*, **14**, 2067.
8. G. Lhoest, R. De Neys, N. Defay, J. Seibl, J. Pecher, and R.H. Martin, *Bull. Soc. Chim. Belge*, **74**, 534 (1965).
9. M. Gorman and J. Sweeny, *Tetrahedron Lett.*, 3105 (1964).
10. M.P. Cava, S.K. Talapatra, J.A. Weisbach, B. Douglas, and G.O. Dudeck, *Tetrahedron Lett.*, 53 (1969).
11. E. Wenkert, C.J. Chang, H.P.S. Chawla, D.W. Cochran, E.W. Hagaman, J.C. King, and K. Orito, *J. Am. Chem. Soc.*, **98**, 3645 (1976).
12. E. Bombardelli, A. Bonati, B. Gabetta, E. Martinelli, G. Mustich, and B. Danieli, *Phytochemistry*, **15**, 2021 (1976).
13. N. Aimi, K. Yamaguchi, S. Sakai, J. Haginiwa, and A. Kubo, *Chem. Pharm. Bull.*, **26**, 3444 (1978).
14. K. Jewers, D.F.G. Pusey, S.R. Sharma, and Y. Ahmad, *Planta Med.*, **38**, 359 (1980).
15. B.E. Leonard, *J. Pharm. Pharmacol.*, **17**, 788 (1965).
16. B.E. Leonard, *J. Pharm. Pharmacol.*, **17**, 566 (1965).
17. F. Sandberg, R. Verpoorte, and A. Cronlund, *Acta Pharm. Suec.*, **8**, 341 (1971).
18. "Pharmacological experiments on isolated preparations," Staff Dept. of Pharmacology, Edinburgh University, Livingstone, 1968, pp 30-37.

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