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9-METHOXYGEISSOSCHIZOL, AN ALKALOID FROM BARK OF *STRYCHNOS GUIANENSIS*

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Key Word Index—*Strychnos guianensis*; Strychnaceae; stem bark; indole alkaloid; curare; 9-methoxygeissoschizol; 2D-NMR.

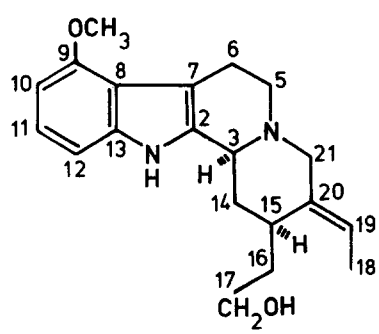
Abstract—The isolation and structural determination of 9-methoxygeissoschizol from the stem bark of *Strychnos guianensis* is described. Elucidation of its structure is mainly based on NMR studies. Copyright ©1996 Elsevier Science Ltd

INTRODUCTION

In the course of our phytochemical investigations of the bark of *Strychnos guianensis* [1], the first botanically identified plant source of South American curare, we describe the isolation and structural determination of a new tertiary alkaloid, 9-methoxygeissoschizol (**1**).

RESULTS AND DISCUSSION

9-Methoxygeissoschizol is a tertiary indole alkaloid giving on TLC with ferric chloride reagent a grey coloration turning to yellow–green. Its UV spectrum shows maxima at 224, 269 and 292 nm, suggesting the chromophore of an indole nucleus [2]. No intense bathochromic shift is observed in alkaline solutions, indicating the absence of any phenolic group. No carbonyl band is observed in its IR spectrum. The electrospray mass spectrum gave a quasi $[M]^+$ peak at *m/z* 327 ($[M+1]^+$) consistent with the elemental composition $C_{20}H_{26}N_2O_2$, of which the structure was mainly deduced from ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum, we observed three aromatic protons whose chemical shifts and couplings are indicative of a 9- or 12-substituted indole nucleus. A one proton quartet was observed at 5.48 ppm, coupled in the COSY spectrum with a three-proton doublet at 1.6 ppm, thus suggesting the presence of an ethylidene side-chain. This proton was also weakly coupled (COSY) with one of the protons of a methylene group at 3.6 and 3 ppm, which corresponds to $C(21)H_2$. A three-proton singlet at 3.87 ppm indicated the presence of a methoxyl group, probably substituting the 9- or 12-position of the aromatic nucleus. The 2D-COSY spectrum also allowed us to deduce two other spin systems. First, a CH_2-CH_2 unit related to the protons at 2.96 (2H), 3.15 and 3.02 ppm. The chemical shift of the last two protons is in agreement with a linkage to a nitrogen atom. This fragment probably arises from the tryptamine moiety and should correspond to N-$C(5)H_2$-$C(6)H_2$. A convenient entry point for the second fragment is provided by the 4.20 ppm broadened singlet which shows a weak coupling with the previous fragment. The connectivities provide the means of assembling the following substructure: CH-CH_2CH-CH_2-CH_2. The chemical shifts of the protons of the last methylene of this fragment (3.52 and 3.57 ppm) are in agreement with linkage to an oxygen atom. Furthermore, the presence of a hydroxyl group was confirmed by acetylation at room temperature. All these data are



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consistent with a 9- or 12-methoxygeissoschizol derivative. The ^{13}C chemical shifts (total decoupling, DEPT and C-H correlation) fully support this structure and allowed us to localize the substituted position on the aromatic ring. Comparison of the observed chemical shifts of C-8, C-9, C-10, C-11, C-12 and C-13 with those calculated using substituent-induced chemical shifts [3] for the corresponding atoms of 18, 19-dihydrocorynantheol [4] indicates a C-9 substitution, as in guianensine [1]. This is further confirmed by the very weak coupling observed between the methoxyl group and H-10 in the COSY spectrum. Therefore, we propose structure **1** for this new alkaloid. All the chemical shifts are collected in Table 1. They fully support this structure, compared with data for known compounds [4, 5].

The stereochemistry of C-3α (*S*) was determined by the positive Cotton effect observed between 270 and 300 nm [6]. The 15α-H (*S*)-configuration agrees with the biogenetic hypothesis [7]. Moreover, its chemical shift (2.89 ppm) confirms this configuration [8]. The orientation of the ethylidenic side-chain is proposed to be *E* because of the chemical shifts observed for C-21 and C-15 [9, 10].

The ^1H NMR spectrum of compound **1** shows broad signal at 4.20 ppm, which is assigned to H-3 characteristic of *cis*-indoloquinolizidine stereochemistry and supported by the lack of Bohlmann bands in the IR spectrum [11]. The ^{13}C signals at δ 53.5, 20.1 and 53.8 assigned to C-3, C-6 and C-21, respectively, are very similar to the equivalent system in 10-methoxygeissoschizol [4], *E*-isositsirikine and 17-deoxy-*E*-isositsirikine [10], which confirm that **1** has the same relative configuration. The indolo [2,3-*a*]-quinolizidine skeleton can exist in three main conformations, owing to nitrogen inversion and *cis*-decalin type ring interconversion.

Table 1. ^1H and ^{13}C NMR data of compound **1** ($CDCl_3$)

H	δ	Correlations*	C	δ
3	4.20 (*br.s.*)	5A, 5B, 14A, 14B	2	131.5†
5A	3.15 (*m*)	3, 5B, 6A, 6B	3	53.5
5B	3.02 (*m*)	3, 5A, 6A, 6B	5	51.1
6A-B	2.96 (*m*)	5A, 5B	6	20.1
10	6.45 (*d*: 8Hz)	11, 12, OMe	7	106.9
11	7.00 (*t*)	10, 12	8	117.4
12	6.93 (*d*: 8Hz)	10, 11	9	154.3
14A	2.26 (*m*)	3, 14B, 15	10	99.6
14B	2.11 (*m*)	3, 14A, 15	11	122.2
15	2.89 (*m*)	14A, 14B, 16	12	104.6
16A-B	1.46 (*m*)	15, 17A, 17B	13	137.5
17A	3.57 (*m*)	16, 17B	14	32.4
17B	3.52 (*m*)	16, 17A	15	31.5
18	1.61 (*t*: 5.5Hz)	19, 21A, 21B	16	35.8
19	5.5 (*q*)	18, 21B	17	61.4
21A	3.6 (*d*: 14 Hz)	18, 21B	18	12.9
21B	3.00 (*m*)	18, 19, 21A	19	121.6
NH	8.70 (*s*)		20	135.9*
OMe	3.87 (*s*)	10	21	53.8
OH	2.81		OMe	55.2

*Observed by means of 2D-COSY spectrum.

†These values may be interchanged.

To avoid a strong interaction between C-19-CH_3 and C-15 side-chain, there would be the preponderance of a 3α-*cis* conformation with an axial C15 side-chain as in geissoschizol [12] and in *E*-isositsirikine [10] derivatives. This conformation would explain the absence of Bohlmann bands in the IR spectrum of compound **1**.

Conversely, 9-substituted indole alkaloids are not common in *Strychnos* species; up to now, only four species exhibit this type of substitution (*S. guianensis* and *S. rubiginosa* from America and *S. lucens* and *S. madagascariensis* from Africa) [1, 13, 14].

EXPERIMENTAL

Plant material. Stem bark of *S. guianensis* (Aubl.) Mart. was collected in April 1988 by L.A. and M-L.-B-P. at Manaus, near Rio Taruma in Amazonia (Brazil). Identification of the plant was confirmed by Dr A. J. M. Leeuwenberg (Wageningen); voucher specimens (INPA Herb. no. 150,295) are deposited not only in the INPA at Manaus, but also in the Pharmaceutical Institute at Liège and the Agricultural University at Wageningen (The Netherlands).

Extraction and isolation. Powdered bark was extracted as described in ref. [1]. The $CHCl_3$ pH8 extract was fractionated first by MPLC on silica gel 60 (Superformance®) using CHC_3–MeOH mixt (19:1, 23:2, 9:1, 4:1, 1:1), then pure MeOH. Frs containing 9-methoxygeissoschizol were then purified on a Fractogel® TSK HW 40S column with EtOH as solvent and finally by TLC on silica gel (solvent systems: EtOAc-isoPrOH-NH_4OH 25%, (945:4:1 and 16:3:1).

Methoxygeissoschizol (**1**). UV λ_{max} (MeOH) (log ε): 210 (sh) (4.21), 224 (4.31), 269 (3.74), 292 (3.6); (MeONa) λ_{max} (log ε) 215 (4.32), 224 (3.27), 271 (3.76), 292 (0.71). CD (MeOH; *c* 0.05): $\Delta\varepsilon_{221} = -1.63$, $\Delta\varepsilon_{269} = +0.52$. ESI-MS (50 eV) *m/z* (rel. int.): 327 (100), 326 (15.8), 325 (4.2), 297 (2.6), 264 (1.4), 214 (1.8), 174 (42.6), 154 (5.8). FTIR ν_{max}^{KBr} (cm^{-1}): 3242, 2926, 2853, 1621, 1592, 1569, 1511, 1437, 1384, 1355, 1251, 1168, 1141, 1105, 1059, 978, 815, 775, 733, 651, 614, 571, 526, 465. ^1H and ^{13}C NMR: Table 1.

Acetylation. To 1 mg of **1** was added 1 ml pyridine and 0.2 ml Ac_2O. After standing 14 hr at room temp., reagents were removed by evapn to yield the monoacetylated compound with a $[M]^+$ peak at *m/z* 369 ($[M + 1]^+$, (ESI-MS).

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REFERENCES

1. Quetin-Leclercq, J., Llabrès, G., Warin, R., Belem-Pinheiro, M.-L., Mavar-Manga, H. and Angenot, L. (1995) *Phytochemistry* **40**, 1557.
2. Scott, A. I. (1964) in *Interpretation of the UV Spectra of Natural Products*, p. 172. Pergamon Press, Oxford.
3. Verpoorte, R., van Beek, T. A., Riegman, R. L. M., Hylands, P. J. and Bisset, N. G. (1984) *Org. Magn. Res.* **22**, 328.
4. Nunes, D. S., Koike, L., Taveira, J. and Reis, F. A. M. (1992) *Phytochemistry* **31**, 2507.
5. Feng, X. Z., Kan, Ch., Potier, P., Kan, S.-K. and Lounasmaa, M. (1982) *Planta Med.* **44**, 212.
6. Toth, G., Hetenyi, F., Clauder, O. and Kajtar, M. (1978) *Liebigs Ann. Chem.* 1096.
7. Klyne, W. and Buckingham, J. (1974) *Atlas of Stereochemistry—Absolute Configuration of Organic Molecules*. Chapman and Hall, London.
8. Gribble, G. W., Nelson, R. B., Johnson, J. L. and Levy, G. C. (1975) *J. Org. Chem.* **40**, 3720.
9. Aimi, N. Yamaguchi, K., Sakai, S.-I., Haginiwa, J. and Kubo, A. (1978) *Chem. Pharm. Bull.* **26**, 3444.
10. Lounasmaa, M., Jokela, R., Hanhinen, P., Miettinen, J., and Salo, J. (1994) *Tetrahedron* **50**, 9207.
11. Trager, W. F., Lee, C. M. and Beckett, A. H. (1967) *Tetrahedron* **23**, 365.
12. Dastoor, N. S., Gorman, A. A., and Schmid, H. (1967) *Helv. Chim. Acta* **50**, 213.
13. Marini-Bettolo, G. B., Galeffi, C., Nicoletti, M. and Messena, I. (1980) *Phytochemistry* **19**, 992.
14. Delaude, C., Thépenier, P., Jacquier, M. J. Massiot, G. and Le Men-Olivier, L. (1992) *Bull. Soc. Roy.*