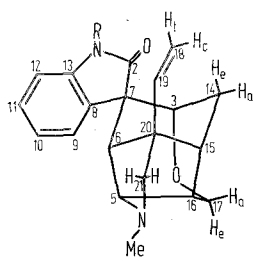
EXPERIENTIA

Vol. 28 – Fasc. 4 Pag. 377–496 15. 4. 1972

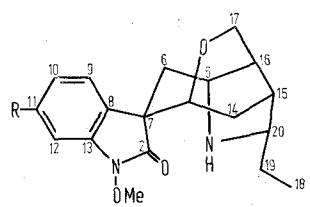
SPECIALIA

The Structure of Gelsevirine[1]

Some time ago the biosynthetically unusual alkaloids sempervirine, gelsemine (**1a**), gelsedine (**2a**), gelsemicine (11-methoxygelsedine) (**2b**) and gelsevirine were isolated from the roots of yellow jasmine (*Gelsemium sempervirens* Ait.)[2]. Since only the minor oxindole alkaloid gelsevirine ($C_{21}H_{24-26}O_3N_2$) remained of undetermined constitution, its structure analysis was undertaken. In the process high resolution mass spectrometry, and ^1H and ^{13}C NMR spectroscopy were employed. As a consequence the use of these powerful, new, analytical methods was extended to a thorough examination of structurally highly complex gelsemine and gelsedine.



1a, R = H
b, R = OMe
c, R = Me



2a, R = H
b, R = OMe

The molecular ion peak of 352 of the oily minor base, identified as MARION'S[2] gelsevirine by comparison of its IR- and UV-spectra and its methiodide melting point with the reported physical constants, proved it to be a $C_{21}H_{24}O_3N_2$ substance. Intense 9.2–9.4 μm IR-absorption and a 3.91 ppm PMR three-proton singlet revealed it to possess a methoxy group and general similarity of all its spectra with those of gelsemine (**1a**) suggested gelsevirine might be methoxygelsemine.

The mass spectrum of gelsemine (**1a**) exhibited a base peak (M-214), characteristic of fragmentation (a), metastable M-43 and M-71 peaks (m* 241.7 and 225.8, respectively), indicative of path (b), and a M-202 peak, probably due to the formation of the 1-methyl-3-vinylpyridinium ion[3]. Gelsevirine showed the same fragmentation pattern along with additional peaks involving the loss of the methoxy group. A metastable M-31 peak (m* 291.8) corresponding to a methoxyl loss from the molecular ion was followed by a metastable M-31-30 peak (m* 264) due to the loss of nitric oxide from the M-31 oxindole moiety. The metastable M-43 peak (m* 271.3), a result of gelsevirine's equivalent of gelsemine's fragmentation (a), also suffered loss of the methoxyl group (M-74). These methoxyl extrusions were reminiscent of the behavior of the N_a-methoxy unit of gelsedine (**2a**). The mass spectrum of the latter reveals metastable M-31 (m* 272.6) (methoxy loss), M-29 (m* 268.9) (ethyl loss) and M-29-31 (m* 240.2) (methoxyl loss from the M-29 fragment) peaks. Loss of a C_5H_6O fragment from the molecular ion gave a M-82 peak from which emanated a metastable M-82-31 peak (m* 187.9) representing one more methoxyl extrusion. The base peak was M-176, a probable consequence of transformation (c). All these data pointed to gelsevirine being N_a-methoxygelsemine (**1b**).

The 220 MHz PMR spectra of gelsemine (**1a**)[4] and gelsevirine were nearly identical except for the chemical shift of H-12 (Table I). A 220 MHz PMR spectrum of gelsedine (**2a**) revealed an extraordinary similarity of its aromatic region [H-9 7.35 (d, J = 7.5 Hz), H-10 7.06 (t, J = 7.5 Hz), H-11 7.24 (t, J = 7.5 Hz), H-12 6.90 ppm (d, J = 7.5 Hz)] and its methoxyl signal (3.96 ppm) with those of the gelsevirine spectrum[5]. These facts support structure **1b** for gelsevirine.

Comparison of the CMR spectrum of gelsevirine with the spectra of gelsemine (**1a**)[6] and N_a-methylgelsemine

[1] This communication represents paper VIII of the series 'Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances'. For the preceding article see E. WENKERT, D. W. COCHRAN, E. W. HAGAMAN, R. B. LEWIS and F. M. SCHELL, J. Am. chem. Soc. *93*, 6271 (1971).

[2] H. SCHWARTZ and L. MARION, Can. J. Chem. *31*, 958 (1953) and references therein.

[3] The authors are indebted to the Mass Spectrometry Laboratory of the Batelle Memorial Institute for the high-resolution spectrum and element map.

[4] For a description of a 60 MHz PMR spectrum of gelsemine see H. CONROY and J. K. CHARKABARTI, Tetrahedron Lett. *4*, 6 (1959).

[5] The remainder of the spectrum of gelsedine (**2a**), except for the oxymethylene signal [4.19 (d, J = 11.0 Hz), 4.27 (dd, J = 11.0, 4.0 Hz)] was nearly as ambiguous as the reported 60 MHz spectrum [E. WENKERT, J. C. ORR, S. GARRATT, J. H. HANSEN, B. WICKBERG and C. L. LEICHT, J. Org. Chem. *27*, 4123 (1962)].

[6] E. WENKERT, C.-J. CHANG, A. O. CLOUSE and D. W. COCHRAN, Chem. Commun. *1970*, 961.

(**1c**)[7] showed the extra methoxyl group to affect the chemical shifts of the carbons of mainly the oxindole subunit and the vinyl group, its 1,3-diaxially oriented neighbor[8] (Table II). The extraordinarily deshielded position of the methoxy group, when compared with methyl esters and methyl ethers of the aliphatic and aromatic types, and its nearly identical δ value with that of gelsedine's methoxyl function confirms the **1b** designation for gelsevirine. Chemical shift assignments of the remaining carbons of gelsedine (**2a**) followed the arguments in the CMR analysis of gelsemine[6].

In analogy with the demethoxylation of gelsedine[5] a reduction of gelsevirine (**1b**) was undertaken. A solution of 120 mg of gelsevirine in 1 ml of methanol was added dropwise to a solution of 25 mg of lithium in 8 ml of liquid ammonia. The colorless solution was poured immediately into a saturated ammonium chloride solution and the mixture extracted with ethyl acetate. Evaporation of the extract, chromatography of the residue, 80 mg, on Florisil and elution with 30:1 chloroform-methanol yielded 35 mg of gelsemine. Thus all facts prove gelsevirine to possess structure **1b**.

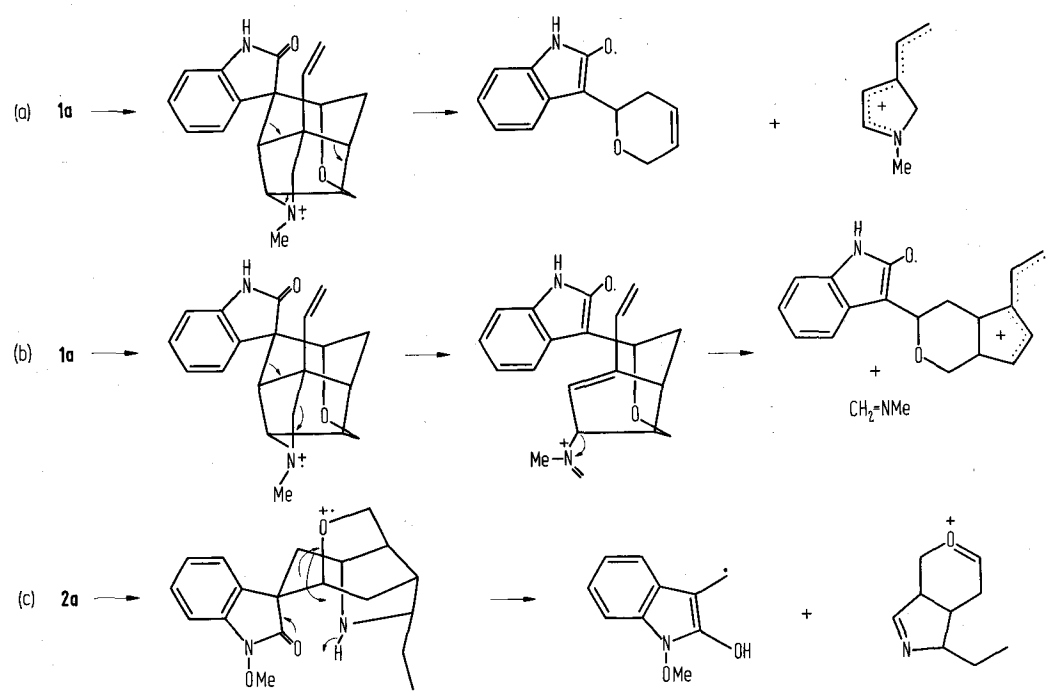


Table I. Chemical shifts (δ in ppm), multiplicities and coupling constants (J in Hz) of PMR spectra of deuteriochloroform solutions of gelsemine (**1a**) and gelsevirine (**1b**).

	Gelsemine		Gelsevirine	
	δ	J[a]	δ	J
H-3	3.79 (d)	2.8	3.78 (d)	2.5
H-5	3.47 (s)	—	3.38 (s)	—
H-6	1.97 (s)	—	1.93 (s)	—
H-9	7.43 (dd)	7.5, 2.0	7.44 (d)	7.5
H-10	6.97 (ddd)	7.5, 7.5, 2.0	7.03 (dd)	7.5, 7.5
H-11	7.15 (ddd)	7.5, 7.5, 2.0	7.26 (dd)	7.5, 7.5
H-12	6.65 (dd)	7.5, 2.0	6.93 (d)	7.5
H_a-14	2.37 (d)	8.0	2.42 (d)	8.0
H_e-14	ca. 2.0 (d)	8.0	1.92 (d)	8.0
H-15	2.83 (dd)	14.5, 2.8	2.81 (dd)	14.0, 2.5
H-16	ca. 2.3 (ddd)	14.5, 2.0, 2.0	2.26 (ddd)	14.0, 2.0, 2.0
H_a-17	3.91 (dd)	11.0, 2.0	3.87 (dd)	11.0, 2.0
H_e-17	4.10 (dd)	11.0, 2.0	4.07 (dd)	11.0, 2.0
H_e-18	4.95 (dd)	18.0, 1.8	4.94 (d)	18.0
H_t-18	5.09 (dd)	11.0, 1.8	5.11 (d)	11.0
H-19	6.28 (dd)	18.0, 11.0	6.19 (dd)	18.0, 11.0
(exo) H-21	2.78 (d)[b]	10.0	2.75 (d)[b]	11.0
(endo) H-21	2.32 (d)[b]	10.0	2.30 (d)[b]	11.0
NMe	2.24 (s)	—	2.23 (s)	—
OMe	—	—	3.91 (s)	—

[a] All couplings < 3 Hz are from a 100 MHz spectrum. [b] These values within any column may be reversed.

Table II. CMR chemical shifts (in ppm upfield from carbon disulfide, $\delta_{CS_2} = \delta_{CHCl_3} + 115.2$ ppm) of chloroform solutions of gelsemine (**1a**) (0.3 *M*), gelsevirine (**1b**) (0.3 *M*), N_a-methylgelsemine (**1c**) (1.0 *M*) and gelsedine (**2a**) (0.3 *M*).

	1a	1b	1c	2a
C-2	13.1	19.3	15.6	17.7
C-3	122.9	122.9	122.9	117.8
C-5	120.4	120.0	120.3	126.8[b]
C-6	151.9	151.8	151.8	158.4
C-7	138.4	140.0	138.7	139.4
C-8	60.3	64.2	61.1	60.4
C-9	64.4[a]	64.2	64.4	66.9
C-10	70.7	69.7	70.6	68.7
C-11	64.1[a]	64.2	64.4	64.3
C-12	83.4	85.1	84.9	85.2
C-13	51.8	52.7	49.2	54.1
C-14	169.5	169.2	169.7	170.9
C-15	154.3	154.3	154.3	157.6[b]
C-16	156.5	156.2	156.7	150.4[b]
C-17	131.0	130.9	131.0	128.5
C-18	80.2	79.3	80.6	180.4
C-19	53.6	54.0	53.4	170.9
C-20	138.4	138.2	138.5	132.7[b]
C-21	126.2	126.1	126.2	—
NMe	141.7	141.2	141.9	—
N_aMe	—	—	166.4	—
OMe	—	129.3	—	129.0

[a] These values may be reversed. [b] The values of C-5 and C-20 and/or those of C-15 and C-16 may need to be interchanged. No models for the strained pyrrolidine unit[9] were available.

Zusammenfassung. Die Massenspektren und ^{1}H- und ^{13}C-NMR-Spektren der Gelsemium-Alkaloide Gelsemin, Gelsedin und Gelsevirin wurden aufgenommen und vollständig analysiert. Gelsevirin wurde durch Reduktion in Gelsemin übergeführt und besitzt die Struktur des N_a-Methoxygelsemins.

E. WENKERT, C.-J. CHANG, D. W. COCHRAN[10] and R. PELLICCIARI

Department of Chemistry, Indiana University, Bloomington (Indiana 47401, USA),
1 October 1971.

[7] C. W. MOORE, J. chem. Soc. *99*, 1231 (1911). – R. GOUTAREL, M.-M. JANOT, V. PRELOG and R. P. A. SNEEDEN, Helv. chim. Acta *34*, 1962 (1951). – V. PRELOG, J. B. PATRICK and B. WITKOP, Helv. chim. Acta *35*, 640 (1952).

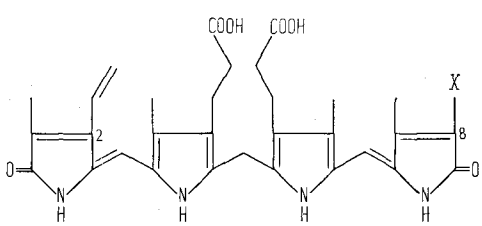
[8] On the assumption of Lewis acid-base complexation affecting the chemical shifts the previous CMR spectrum of gelsemine (**1a**)[6], run at ca. 1.5 *M* concentration, was not used for the comparison study. Further, the former spectrum was recorded on a continuous wave spectrometer, while the present study utilized a Fourier Transform spectrometer.

[9] For an X-ray analysis of gelsemicine (**2b**) see M. PRZYBYLSKA and L. MARION, Can. J. Chem. *39*, 2124 (1961). – M. PRZYBYLSKA, Acta Crystallogr. *15*, 301 (1962).

[10] U.S. Public Health Service predoctoral fellow, 1967–1971.

Photoaddition of Sulphydryl Groups to Bilirubin in vitro

While investigating the role of light in lowering the serum bilirubin level of infants with neonatal hyperbilirubinemia, one of us found that in vitro photochemical addition of alcohols to the *exo*-vinyl group of bilirubin (I) gave rise to products such as II[1]. We now report that compounds containing a sulfhydryl group also undergo analogous regio-specific[2] photoaddition to bilirubin both in chloroform and aqueous solutions.



I X = $-CH=CH_2$
II X = $-CH(CH_3)-OR$
III X = $-CH(CH_3)-SCH_2COOCH_3$
IV X = $-CH(CH_3)-SCH_2CH_2OH$
V X = $-CH(CH_3)-OCH_2CH_2SH$
VI X = $-CH(CH_3)-SCH_2CH(NHCOCH_3)COOH$
VII X = $-CH(CH_3)-SCH_2CHCONHCH_2COOH$
$NHCOCH_2CH_2CH(NH_2)COOH$

When bilirubin dissolved in chloroform (1 mg/ml) containing 5% (v/v) methyl thioglycollate was exposed to UV-light[3], disappearance of the starting material was complete in ca. 1 h and accompanied by the formation of a new compound migrating just above bilirubin in TLC[3]. The yellow photoproduct was then obtained pure on TLC from the residue of evaporation of the reaction mixture after all the green biliverdinoid by-products had been removed by washing with methanol [45% yield; crystallised from $CHCl_3$–CH_3OH 1:2[4]; $\lambda_{max}^{CHCl_3}$ 449 nm (ε 56,000); ν_{max} 3410, 3260, 1735, 1695, 1650, 1615 cm^{-1} (in $CHCl_3$)]. Structure III was assigned to this photoderivative of bilirubin on the basis of its elemental analysis[4] and of its NMR-spectrum[5], which exhibited (in $CDCl_3$) the characteristic ABX signals of the vinyl group at position 2 (*endo*) in the biladiene-*a,c* skeleton[1] (δ_A, δ_B, δ_X = 5.53, 5.39, 6.60 and J_{AX}, J_{BX}, J_{AB} = 18.0, 11.1, 1.4 Hz) and further a set of peaks associated with the grouping $-CH(CH_3)SCH_2COOCH_3$ [1.57$_d$ (3H, J = 7 Hz, $-CH_3$), 3.14$_s$ (2H, $-CH_2-$), 3.66$_s$ (3H, $-OCH_3$) and 4.02$_q$ (1H, J = 7 Hz, >CH-S-)[6]].

[1] P. MANITTO, Experientia *27*, 1147 (1971).

[2] A. HASSNER, J. org. Chem. *33*, 2684 (1968).

[3] Irradiations were conducted as described in ref.[1]; thin-layer chromatography was carried out on polyamide [methanol – 10% ammonia 9:1 (v/v)], spraying the plates with diazotised sulphanilic acid in HCl dil.

[4] All the new compounds blackened without melting over 250°; they gave correct elemental analyses consistent with the assigned structures.

[5] Chemical shifts are in parts per million (δ) from internal tetramethylsilane; *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet.

[6] Partly buried beneath the signals of the central methylene bridge.