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.

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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).

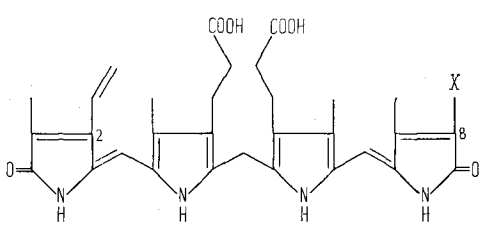
[8] On the assumption of Lewis acid-base complexation affecting the chemical shifts the previous CMR spectrum of gelsemine (**1a**)[6], run at ca. 1.5 *M* concentration, was not used for the comparison study. Further, the former spectrum was recorded on a continuous wave spectrometer, while the present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

Likewise, irradiation of bilirubin in chloroform containing 2-mercaptoethanol [5% (v/v)] or N-acetyl-L-cysteine (2 mg/ml, i.e. a saturated solution) led to isolate, after working as described above, the adducts IV (62% yield; crystallised from $CHCl_3$–CH_3OH 1:2[4]) and VI (74% yield; purified by precipitation with HCl dil. from 0.1 *N* NaOH solution[4]), respectively. These compounds showed the following spectroscopic properties consistent with the expected structures: IV, $\lambda_{max}^{CHCl_3}$ 449 nm (ε 53,300); ν_{max} 3420, 3265, 1700, 1650, 1618 cm^{-1} (in $CHCl_3$); NMR ($CDCl_3$)[5], 1.56_d (3H, J = 7 Hz, $-CH_3$); 2.64_t (2H, J = 6 Hz) and 3.70_t (2H, J = 6 Hz) ($-S-CH_2-CH_2-OH$, the hydroxyl proton falling into the range 2.5–3.0); 4.06_q (1H, J = 7 Hz, >CH–S–)[6] and the ABX system of the *endo*-vinyl group (δ_A, δ_B, δ_X = 5.54, 5.40, 6.61 and J_{AX}, J_{BX}, J_{AB} = 17.0, 11.0, 1.4 Hz); VI, λ_{max} 446 nm (ε 52,000) in 6×10^{-4} N methanolic NaOH; ν_{max} 3400, 3260, 1690, 1645, 1610 cm^{-1} (Nujol); NMR (DMSOd_6)[5] 1.50_d (3H, J = 7 Hz, $-CH_3$), 1.85_s (3H, CH_3CO-), 2.71_d (2H, $-S-CH_2-$, doublet partly buried beneath the signals of the ethylene protons of propionic acid side chains), 4.00_q (1H, J = 7 Hz, >CH–S–)[6], 4.35_m (1H, >CH–NH–), the ABX system of the *endo*-vinyl group (δ_A, δ_B, δ_X = 5.60, 5.55, 6.80 and J_{AX}, J_{BX}, J_{AB} = 17.5, 11.0, 1.4 Hz) and 8.19_d (1H, $-NH-COCH_3$).

The fact that IV was formed as essentially the only product of addition[7], and that photoadditions to the *exo*-vinyl group of bilirubin appear to be faster with thiols than with alcohols, is understandable if one takes into account the mechanism proposed for such additions[1] and the difference in nucleophilicity between sulfhydryl and alcoholic function, the former being generally more nucleophilic[8].

It is noteworthy that compound VI was also produced in moderate yield when bilirubin was irradiated in aqueous solution (1 mg/ml in NaOH–KH_2PO_4 adjusted to pH 9.0) in the presence of N-acetyl-L-cysteine (4 mg/ml) for 7–8 h[9]. In addition, TLC evidence was obtained for the formation of a photoadduct of bilirubin with glutathione (likely VII) by irradiation of a mixture of these substances in aqueous solution (1 mg/ml of bilirubin and 2 mg/ml of GSH in NaOH–KH_2PO_4 adjusted to pH 9.0).

All the above findings support our hypothesis[1] that at least part of the serum bilirubin in animals and humans exposed to natural and artificial light is eliminated as photoadducts with nucleophilic substances, for instance GSH and, by implication, albumin. The last one seems particularly appropriate to give an irreversible adduct with bilirubin: in fact, it is well recognized that a reversible albumin-bilirubin complex[10] occurs in the extracellular fluids of the body; furthermore, it has been reported that the reactive thiol group of native albumin is contained in the loosely organized portion of the protein which is probably involved in binding a great many compounds of biological importance[11].

The clinical implications of our results are being investigated further[12].

Riassunto. Viene descritta l'addizione fotochimica dei tioli al doppio legame *eso* della bilirubina. Si prospetta l'ipotesi che una reazione di questo tipo sia responsabile della rapida riduzione della bilirubinemia nei neonati itterici sottoposti a fototerapia.

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13 October 1971.

[7] No isomer of IV, particularly V, was detected in the irradiation mixture.

[8] C. K. INGOLD, *Structure and Mechanism in Organic Chemistry*, 2nd ed. (Cornell University Press, Ithaca and London 1969), p. 451.

[9] It was isolated from the irradiated solution made acid with HCl dil. and shown to be identical, byTLC, IR- and NMR-spectra, with compound VI obtained previously.

[10] N. H. MARTIN, J. Am. chem. Soc. *71*, 1230 (1949).–D. BRATLID and J. FOG, Scand. J. clin. Lab. Invest. *25*, 257 (1970) and references cited therein. – G. B. ODELL, Pediatrics *46*, 16 (1970).

[11] G. FRANGLEN and G. R. E. SWANIKER, Biochem. J. *109*, 107 (1968).

[12] The authors wish to thank Prof. L. CANONICA for helpful discussion and Dr. G. SEVERINI RICCA for running NMR-spectra.

Isolation of Quercetin 3,7,3',4'-Tetrasulphate from *Flaveria bidentis* L. Otto Kuntze

In the course of an investigation of the flavonoids present in the leaves of *F. bidentis* (Compositae), which were collected from plants growing in the central part of Argentina, a new compound was isolated from the methanol-water (50%) extracts, which was characterized as quercetin 3,7,3',4' tetrasulphate. The pure crystalline sodium salt was obtained by passing a solution of the crude crystalls through a column of Amberlite 120 (H), and then through Amberlite ICR 50 (Na). The salt carried without melting at 360°.

Found: C, 25.98; H, 1.18; SO_4, 53.54; S, 17.88; Na, 12.98; Cal. for $C_{15}H_6O_{19}S_4Na_4$: C, 25.36; H, 0.85; SO_4, 54.08; S, 18.05; Na, 12.94%. UV: (EtOH); λ_{max}. 270; 310; 340 sh nm (log ε, 4.32; 4.09; 4.04). IR: strong band at 3531 cm^{-1} (OH). NMR: (D_2O; 60 MHz) 6.78 (1 H, d, $J_{6,8}$ = 2.5 Hz, C6–H); 7.13 (1 H, d, $J_{8,6}$ = 2.5 Hz; C8-H); 7.66 (1 H, d, $J_{5',6'}$ = 8.5; C5'–H); 7.96 (1 H, d, $J_{2',6'}$ = 2.5; C2'–H); 8.16 (1 H, br. sig. C6'–H).

Chromatography; Whatman 1. Rf AcOH 27%-n-BuOH (1:1) 0.50; $CH_3COOH-H_2O$ (60:40) 0.75; H_2O, 0.92.

Hydrolysis with 0.1 *N* HCl at 100°, produced crystals, m.p. 312–313° (dec.) which were identified as quercetin by UV[1]-, IR-spectra and Rf values on paper chromatography, employing 5 different systems.

The isolated tetrasulphate was methylated in dimethylsulphoxide solution with diazomethane in ether. Working of the reaction product in the usual way, gave a white crude crystalline solid with an IR-spectrum lacking the

[1] K. PAECH and M. V. TRACEY, *Modern Methods of Plant Analysis* (Springer-Verlag, Berlin 1955), vol. 3, p. 476.