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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[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.

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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.

OH band. It was hydrolized as described for the parent compound, and 5 *O*-methylquercetin (azaleatine)[2], isolated and characterized by comparison with an authentic sample. UV-, IR-spectra and Rf values, on paper chromatography (2 systems) were identical.

Quercetin-3, 7, 3′, 4′-tetrasulphate is the first flavonoid polysulphate isolated from plants and the first found in a species of the Compositae.

Two monosulphates of flavonoids, isorhamnetin-3-sulphate (persicarin) and rhamnazin-3-sulphate have been isolated from a few species of plants belonging to the genus *Polygonum* (Polgonaceae) and *Oenanthe* (Umbelliferae)[3]; Tamarixin, 4 O-methyl quercetin-3-sulphate has been found in *Tamarix troupii*[4] and *T. laxa*[5] (Tamaricaceae). Synthetic sulphonated flavonoids have also been described[6].

Résumé. A partir de l'extrait des feuilles de *Flaveria bidentis* (Compositae), on peut obtenir le 3, 7, 3′, 4′ tétrasulphate de quercétine. Sa constitution a été déterminée par des méthodes physiques et chimiques.

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Butterfly Wing Antineoplastic Agents[1,2]

The colorful pigmentation of butterflies became an early object of scientific inquiry[3,4]. Fortunately, these early studies of butterfly wing constituents provided a foundation in pteridine chemistry which allowed more rapid structural elucidation of folic acid and synthesis of the clinically useful cancer chemotherapeutic agent methotrexate. For the purpose of locating potentially useful antitumor agents among animal constituents, we have undertaken an extensive survey of terrestrial[4] and marine[1] arthropods. Initial studies[4] indicated that the insect order Lepidoptera and particularly several members of the Pieridae family of butterflies warranted detailed investigation. We now wish to report results from the first chemical examination of a butterfly, and in fact of an arthropod, for antitumor constituents.

The yellow Asian butterfly *Catopsilia crocale* Cramer (Pieridae) was extracted consecutively with ligroin, 50% ethanol and 95% ethanol. The latter extract reached the confirmed active stage (71% inhibition of tumor growth at 400 mg/kg) in the National Cancer Institute's Walker 256 carcinoma (s.c. in random-bred albino rats) tumor system. A vigorous effort at recollection at times involving up to 500 field collectors eventually provided 250,000 members of this species. Dissection into head, thorax, abdomen and wing parts followed by re-extraction and biological evaluation of each section established that the antineoplastic component(s) was distributed more or less throughout the butterfly, but principally in the wing material. A 1517 g amount of *Catopsilia crocale* Cramer wings led to 50, 51 and 53 g quantities respectively of ligroin, 50% ethanol and 95 %ethanol extracts. Separation of the 95% ethanol extract was directed by means of bioassay (Walker 256 carcinoma). The crude material was partitioned consecutively between water-chloroform-*n*-butanol (1:1:0.1), water-*n*-butanol (1:1) and water-methanol-*n*-butanol (1:0.25:1). Antitumor activity was shown to reside in the methanol-*n*-butanol extract and in the water phase. After an extensive series of column (Sephadex G-10 and cellulose) and preparative thin layer chromatographic separations dictated by results of bioassay, a substantial portion of the antitumor activity was attributed to isoxanthopterin[3,5] (Ia, 71% inhibition of tumor growth at 90 mg/kg) in the water phase.

While isoxanthopterin (Ia) also appeared to account for a majority of the antitumor activity of *Pieris rapae cruavora*[4], its presence could not be substantiated in another active Pieridae, *Prioneris thestylis* Dbldy[4,6]. In the work with *Pieris rapae* it was found most convenient to isolate isoxanthopterin by extracting the wings with dilute aqueous ammonia followed by separation using Sephadex G-10 and final removal of isoxanthopterin from xanthopterin (Ib) and other closely related components by ion-exchange chromatography (SP-Sephadex C-25).

[1] The present contribution represents Part XXVII of the series Antineoplastic Agents. For Part XXVI refer to G. R. Pettit, J. F. Day, J. L. Hartwell and H. B. Wood, Nature, Lond. *227*, 962 (1970).
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