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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28 September 1971.

[2] I. Jurd and R. M. Horowitz, J. org. Chem. *22*, 1618 (1957).
[3] W. Karrer, *Konstitution und Vorkommen der Organischen Pflanzenstoffe* (Birkhäuser Verlag, Basel 1958), p. 618.
[4] S. R. Gupta and T. R. Seshadri, J. chem. Soc. *1954*, 3063.
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[6] Part of this research was carried out with funds provided by the Instituto Nacional de Farmacologia y Bromatología (Buenos Aires, Argentina) and is O.J.P. de S.'s doctoral thesis.
[7] Acknowledgments. We thank Professors V. Deulofeu (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires) and R. Caputto (Facultad de Ciencias Químicas, Univ. Nac. de Córdoba) for the very useful discussions. To Agron. A. Hunziker (Museum of Botany, Universidad Nacional de Córdoba) for the taxonomic classification. To Dr. H. M. Horowitz (Pasadena, California) for the authentic sample of Azaleatine.

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).
[2] This investigation was supported by Public Health Service Research Grants No. CA-10612-01 to No. CA-10612-04 from the National Cancer Institute, and was presented in part at the American Chemical Society Meeting, Washington, D.C., September 1971. We are also grateful to the National Science Foundation for financial assistance (Grant numbers GB-4939 and GB-6979) used in obtaining the Atlas CH-4B and SM-1B mass spectrometers employed in this study.
[3] Important reviews of naturally occurring pteridines have been prepared by R. C. Elderfield and A. C. Metha in *Heterocyclic Compounds* (Ed. R. C. Elderfield; John Wiley and Sons, Inc., New York, N.Y. 1967), Vol. 9, p. 1–117 – and W. Pfleiderer, Angew. Chem. (Int. Ed. Engl.), *3*, 114 (1964).
[4] G. R. Pettit, J. L. Hartwell and H. B. Wood, Cancer Res. *28*, 2168 (1968).
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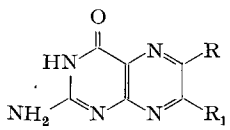
The butterfly pteridines were most conveniently assessed by mass spectral methods. For example, in the case of isoxanthopterin high resolution (Atlas SM-1B) mass determination by peak matching (PFK internal standard) showed the molecular ion as the base peak at *m/e* 179.0443 (calcd. for $C_6H_5N_5O_2$: 179.0443). We have also ascertained, by elemental analysis, that the wings of these particular butterflies contain 4–5% silicon. This intriguing observation warrants further study.

Because the presence of 7-methyl xanthopterin (Ic), pterin 6-carboxylic acid (Id), pterin 7-carboxylic acid (Ie) and erythropterin (If) were suspected during the separation of *Catopsilia crocale* Cramer fractions, synthetic samples of each were obtained and submitted for antitumor evaluation. Both erythropterin and pterin 6-carboxylic acid were found to be marginally inactive, while pteridines Ic and Ie were inactive in analogous dose ranges. Present results suggest that the insect pteridines represent an important starting point for design of potentially useful antineoplastic agents.



Ia, R = H, R_1 = OH
b, R = OH, R_1 = H
c, R = OH, R_1 = CH_3
d, R = CO_2H, R_1 = H
e, R = H, R_1 = CO_2H
f, R = OH, R_1 = CH_2COCO_2H

Zusammenfassung. Eine Voruntersuchung der Insektengruppe Lepidoptera auf anti-tumor-aktive Stoffe führte zu einer detaillierten chemischen Prüfung der aus Asien stammenden Schmetterlinge *Catopsilia crocale* Cramer (Pieridae) und *Pieris rapae cruavora*. Ein bedeutender Teil der Anti-Tumor-Aktivität scheint ihren Ursprung in der chemischen Substanz Isoxanthopterin zu besitzen.

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Department of Chemistry, Arizona State University, Tempe (Arizona 85281, USA); and Drug Research and Development, National Cancer Institute, National Institutes of Health, Bethesda (Maryland 20014, USA), 22 September 1971.

[6] The active constituents of *Prioneris thestylis* are being examined in our laboratory and represent a new series of butterfly components.

A Revision of the Structures of the Lunaria Alkaloids LBX and LBZ

The minor lunaria alkaloids LBX and LBZ, from *Lunaria biennis* Moench, were proposed structures (I) and (II), respectively[1]; on the basis that treatment of lunarine (III) with formaldehyde and dilute acid gave a normal Mannich product, which was identical with alkaloid LBX, while alkaloid LBZ is a corresponding reduction product – one of the epidermic alcohols. The stereochemistry of the alcohol was yet to be determined. We report herein evidence requiring that alkaloid LBX be changed to structure (IV) and alkaloid LBZ to (V). In addition, the configuration of the alcoholic carbon was established as S, according to the Sequence Rule. This removes the last uncertainly about the structure of yet another minor lunaria alkaloid LBY (VI).

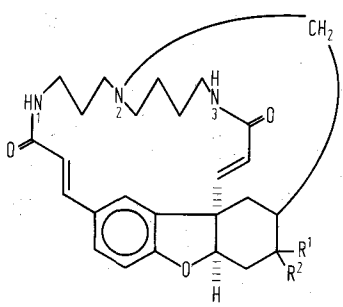
The product[2] (alkaloid LBX) of lunarine (III) and formaldehyde has the following properties which agree only with structure (IV). First, the determined active hydrogen value was 0.99. Second, the NMR-spectrum[3] shows a clean two-proton AB quartet with δ_A 4.16 and δ_B 3.98. (J = 12.5 Hz), while the formaldehyde-d_2-lunarine product [molecular ion[4] at *m/e* 451 (100%)] exhibited an identical spectrum except for the absence of the AB quartet. If the

[1] C. POUPAT, B. RODRIGUEZ, H.-P. HUSSON, P. POTIER and M.-M. JANOT, C. r. Acad. Sci., Paris *269 C*, 335 (1969).

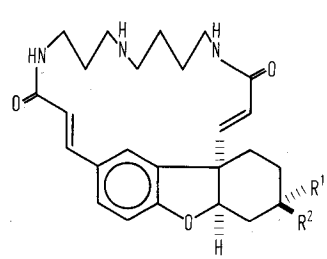
[2] All known compounds mentioned here had physical properties in accord with the literature values, except this substance which gave a higher optical rotation, $[\alpha]_D^{22}$ + 348° (c 0.086, chloroform). The new compounds had elemental analyses or mass spectral data consistent with the proposed structures.

[3] Taken in $CDCl_3$ at 60 MHz with Me_4Si as internal standard.

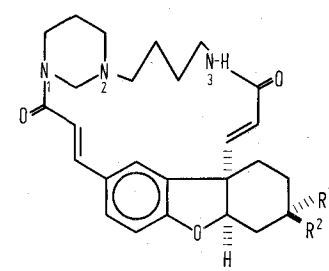
[4] Determined on an AEI MS-9 double-focusing mass spectrometer via direct inlet probe.



(I; $R^1 + R^2 = 0$)
(II; R^1 = H, R^2 = OH)



(III; $R^1 + R^2 = 0$)
(VI; R^1 = OH, R^2 = H)
(VII; R^2 = H, R^2 = OH)



(IV; $R^1 + R^2 = 0$)
(V; R^1 = OH, R^2 = H)
(VIII; R^1 = H, R^2 = OH)