PATULITRIN AND ACETYL PATULITRIN, FLAVONOL GLYCOSIDES FROM *LAGASCEA MOLLIS*

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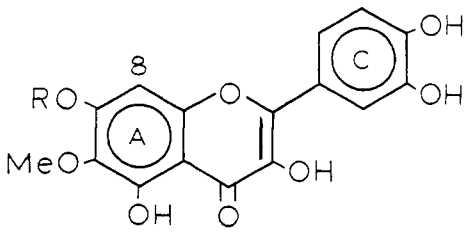
The genus *Lagascea*, family Compositae, is native to Mexico and contains eight species. Little previous phytochemical work has been reported. The polyene ketone, dehydrofalcarinone, was isolated from the roots of *L. mollis* Cav. (1); and an unknown alkaloid, resin, and a fluorescent material were obtained from *L. spinosissima* Cav. (2). We were able to obtain samples of plant material from T. F. Stuessy, of the Botany Department, who has reexamined the classification of this genus (3).

The ethanolic extract residue from the aboveground portion of *Lagascea mollis* Cav. was partitioned between chloroform and water, and the aqueous phase was extracted with ethyl acetate to give a fraction that showed on tlc two major spots exhibiting flavonoid characteristics. Column chromatography separated these substances. The more polar compound was identified as patulitrin (**1**) (4), mp 256–258° on the basis of nmr and ir spectra and the shifts in the uv spectrum in the presence of diagnostic reagents (5). The paper chromatographic mobility was consistent with a 7-*O*-monoglycoside (5); and, on acid hydrolysis, patulitrin (**1**) gave glucose and patuletin (**2**) (4, 6).

The less polar compound was identified as acetyl patulitrin (**3**) on the following evidence. The nmr spectrum in MeOH-d_4 showed to three-proton singlets at δ 2.03 and 3.92 ppm assigned to an acetate methyl and a methoxy, respectively; while the aromatic region integrated for four protons with a pattern[1] typical of flavonols with hydrogens at positions 8, 2', 5' and 6'. The H–8 singlet was located at δ 6.71. The H–5' proton was at δ 8.67 (J=8 Hz), the A part of an ABX pattern; and the H–2' and H–6' protons, its BX portion, were located between δ 7.4–7.8 ppm. The uv spectrum showed a 17 nm bathochromic shift for Band I in the presence of $NaOAc/H_3BO_3$ supporting a 3',4'-di-



1, R = glucosyl

2, R = H

3, R = acetylglucosyl

hydroxy structure. A similar shift of 59 nm in the presence of $AlCl_3/HCl$ was consistent with a 3-hydroxy- or 3,5-dihydroxyflavone. These and other uv spectral characteristics of acetyl patulitrin (**3**) were identical with those of patulitrin (**1**). The ir peak at 1725 cm^{-1} and the nmr peak at δ 2.03 required the acetate to be aliphatic and, thus, located on the sugar unit. The R_F values in standard paper chromatographic systems (3) suggested a 7-*O*-monoglycoside, and acid hydrolysis afforded glucose and patuletin. The former was identified by paper chromatography, and the latter by direct comparison with the aglycone

[1]Spectra No. 51 through 58 of reference (5).

from patulitrin (**1**). Lack of sufficient quantity of acetyl patuletin (**3**) precluded extensive chemical studies to locate the exact position of the acetyl group.

EXPERIMENTAL[2]

PLANT MATERIAL.—The above-ground portion of *Lagascea mollis* Cav. was collected by Prof. T. F. Stuessy, Dept. of Botany, Ohio State University, near Veracruz, Mexico, during September 1973. A voucher specimen is on deposit in the OSU Herbarium (OS) under collection Stuessy and Gardner No. 3184. The air-dried material was powdered to 20 mesh.

EXTRACTION AND FRACTIONATION.—A 1.73 kg sample of powdered tops was percolated at room temperature with ethanol. After removal of the solvent at reduced pressure, the residue (89 g) was partitioned between 1 liter water and 3 x 1 liter of chloroform. The chloroform solubles weighed 33.6 g. Extraction of the aqueous solution with 3 x 1 liter ethyl acetate yielded 2.6 g of extractable material of which 2.5 g was chromatographed on 300 g of silicic acid containing 13.5% water with chloroform-methanol-water (15:3.75:1, lower phase) as solvent system. Column fractions (24 ml) were analyzed by tlc by means of the same solvent system as for column separation but with five repeat developments and detection by spraying with 1% ethanolic $AlCl_3$.

ISOLATION OF PATULITRIN (**1**).—Column fractions with R_F 0.22 material were pooled and the residue (240 mg) crystallized from aqueous MeOH to give yellow prisms of patulitrin (**1**), mp 256–258° (d) [lit. value (4), mp 254–256° (d)]; $[\alpha]^{21}D-78°$ (*c*, 1.0 MeOH); uv λ max 373 nm (log ε 4.27) and 259 (4.36) with changes in the presence of NaOAc-H_3BO_3, $AlCl_3$, and $AlCl_3$-Hcl as reported (6). The nmr spectrum of the trimethylsilyl ether derivative appeared essentially as pictured.[3] Analysis by paper chromatography showed R_F 0.34 in t-butanol-acetic acid-water (3:1:1) and R_F 0.17 in acetic acid-water (15:85), typical of 7-*O*-monoglycosides. Direct comparison (uv and paper chromatography) with an authentic sample confirmed the identity.

[2]Melting points are uncorrected and were determined in capillaries on a Thomas-Hoover Uni-Melt apparatus. Ir spectra were taken on a Beckman model 4230 instrument under stated conditions. Uv spectra were taken in MeOH on a Cary model 15 spectrophotometer. Nmr spectra were taken on a Varian A-60A instrument under stated conditions. Optical rotations were measured on a Perkin-Elmer model 241 photoelectric polarimeter. Flavonoid diagnostic reagents and procedures were according to Mabry *et al.*, (5).

[3]Spectrum No. 51 of reference 5, p. 305.

HYDROLYSIS OF PATULITRIN (**1**).—A 3 mg sample of patulitrin (**1**) in 3 ml of 2N HCl and MeOH to affect solution was heated 45 min. on a steam bath. On cooling, crystalline material (1.7 mg) formed which was recrystallized from aqueous MeOH to give patuletin (**2**) as yellow needles, mp 261° (d) [lit. value (4), mp 260–261°]. Identification was made by comparison of physical properties (uv analysis and nmr) with reported values.

The residue from the hydrolysis filtrate, after passing through a small polyamide column, was analyzed by paper chromatography and found to contain glucose when compared to standard sugars.

ISOLATION OF ACETYL PATULITRIN (**3**).—Column fractions with R_F 0.38 material were pooled and the residue (300 mg) chromatographed on 32 g of Sephadex LH-20[4] with methanol. The yellow residue (120 mg) crystallized from methyl ethyl ketone as green-yellow prisms of acetyl patulitrin (**3**); mp 138° (with effervescence) then melting at 160°; $[\alpha]^{21}D-123°$ (*c*, 1.1 MeOH); R_F 0.51 on Whatman #1 with t-butanol-acetic acid-water (3:1:1), and 0.31 with acetic acid-water (15:85); uv λ max 373 nm (log ε 4.32) and 258 (4.39), with shifts in the presence of the following reagents: NaOMe, 418 and 265; $AlCl_3$, 463 and 275; $AlCl_3$+HCl, 432 and 269; NaOAc, 418 and 261; and NaOAc+H_3BO_3, 390 and 262 nm; and ir (KBr) 3400 (OH), 1735 (C=O of acetate) and 1660 cm^{-1} (C=O of flavonol). The nmr spectrum (MeOH-d_4) δ 2.03 (s, 3H, Ac), 3.92 (s, 3H, OMe), 6.71 (s, 1H, H-8), a 3H complex pattern between 6.7–7.8 ppm for ring C protons, and complex absorption between 3.3 and 5.5 ppm for sugar protons.

HYDROLYSIS OF ACETYL PATULITRIN (**3**).—A 10.6 mg sample of acetyl patulitrin (**3**) in 7 ml 2N HCl was diluted with MeOH to affect solution and heated on the steam bath for 45 min. On cooling, a crystalline product formed that was recrystallized from MeOH-H_2O to give yellow needles of patuletin (**2**), mp 260° (d) [lit. value mp 260–261°] and exhibiting physical properties (ir, nmr and tlc) identical to those of an authentic sample.

The hydrolysis filtrate was passed through a polyamide column (0.7 x 2 cm) and the effluent was collected till neutral, then dried under vacuum. The residue was spotted on Whatman #1 along with standard sugars, irrigated with ethyl acetate-pyridine-water (12:5:4) and zones visualized by *p*-anisidine hydrochloride reagent. The unknown sugar was identified as glucose.

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LITERATURE CITED

1. F. BOHLMANN, C. ARNDT, H. BORNOWSKI, H. JASTROW, and K.-M. KLEINE. 1962. *Chem. Ber.*, **95**, 1320.
2. C. WEHMER. 1931. *Die Pflanzenstoffe*. Fischer, Jeng, 2nd edition, Vol. 2, 1931, p. 1214.
3. T. F. STUESSY, Revision of *Lagascea* (Compositae, Heliantheae). Fieldiana Bot., in press.
4. N. R. BANNERJEE and T. R. SESHADRI. 1956. *Pro. Ind. Acad. Sci.*, **44A**, 284.
5. T. J. MABRY, K. R. MARKHAM and M. B. THOMAS. 1970. *The Systematic Identification of Flavonoids*. Springer-Verlag, New York.
6. M. B. THOMAS and T. J. MABRY. 1968. *Phytochemistry*, *7*, 787.

BOOK REVIEWS

Biosynthetic Products for Cancer Chemotherapy, Volume 1, GEORGE R. PETTIT, Cancer Research Institute, Arizona State University. *Volume 2*, GEORGE R. PETTIT and GORDON M. CRAGG, University of Capetown, South Africa. Plenum Press, 227 West 17th Street, New York, N.Y. 10011, 1977. Volume 1, xii+215 pp, 15.6 x 23.6 cm, $19.50. Volume 2, ix+150 pp, 15.6 x 23.5 cm, $29.50.

The use of plant extracts for the treatment of cancer dates back at least 5000 years, but the systematic investigation of plants and also animal products as cancer chemotherapeutic agents dates only from the 1940's. In the relatively short period since then, almost 100,000 extracts have been tested, and some 400 active constituents have been isolated and identified from those extracts that proved to contain active material. The literature of this vast effort, overlapping as it does the fields of pharmacognosy, organic chemistry, and medicine, has been scattered through many different journals and other publications, and it is thus difficult for any one investigator to keep track of it all. In the first volume under review, Dr. Pettit has drawn together information relating to the biological origin, structure, and anticancer activity of compounds which show *in vivo* activity against cancer. In Volume 2 a tabular summary of all the naturally occuring antineoplastic and cytotoxic substances described in the chemical literature is presented.

The first volume opens with an excellent introductory chapter dealing with the nature and causes of cancer and with current methods for cancer treatment, including chemotherapy using synthetic drugs. The only weakness of this treatment is that some of the problems of cancer chemotherapy, including that of the development of drug resistance, are not mentioned. The second chapter outlines the history of the search for naturally occurring anticancer drugs, with especial reference to the National Cancer Institute's Drug Research and Development program. The bulk of the book is then given over to eight chapters describing anticancer agents isolated from higher plants, from fungi and other lower plants, and from marine and other animal sources. The compounds obtained from higher plants are described by structural type, with separate chapters on terpenoids, steroids, lignans, quinones and other nonnitrogeneous products, and alkaloids and miscellaneous nitrogeneous products. The presentation makes extensive use of structural formulae (almost a half of the space in the book is devoted to structures) and the tremendous diversity of natural products with anticancer activity comes across very clearly in these chapters.

The second volume contains a listing of some 400 compounds that have been isolated and identified as anticancer and cytotoxic substances in literature available to April 1976. The structure of each compound is given, together with pertinent data such as the molecular composition, melting point, organism of origin and (most welcome) biological activity. The compounds are arranged by chemical class, and the volume concludes with a brief discussion of the evaluation systems employed by the U. S. Natural Cancer Institute and compound and organism indexes.

The book was written, according to the preface, "to provide an overall view of the cancer problem and the development of cancer chemotherapeutic drugs of biosynthetic origin." Within this framework, it succeeds very well. One would not turn to it for a detailed discussion of the isolation or mechanism of action of any anticancer drug, but it would be the first place that this reviewer would turn to for a rapid update on the active compounds in a particular area and for leading references to isolation or mechanism of action studies. An extensive set of over 400 references and a useful index enhance the value of the book.

Dr. Pettit has done the scientific community a service in writing this book, and it is sure to find a ready acceptance among all workers in this important area of natural products.

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