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SHORT COMMUNICATION

PHENOLIC COMPOUNDS IN FERNS—II.

INDIRECT EVIDENCE FOR THE EXISTENCE OF 2′,6′-DIHYDROXY-4,4′-DIMETHOXYCHALCONE IN *PITYROGRAMMA CALOMELANOS*

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Abstract—4′,7-Dimethoxy-5-hydroxyflavanone has been identified as a major constituent of the alkali-soluble fraction of the foliar exudate of *Pityrogramma calomelanos* (L.) Link. This compound does not occur naturally in the exudate but is formed by the alkali-catalyzed cyclization of 2′,6′-dihydroxy-4,4′-dimethoxychalcone. This chalcone and compounds with closely related structures have been found in varieties of *P. chrysophylla* suggesting that this substitution pattern may be a useful chemosystematic characteristic for the genus.

INTRODUCTION

IN 1959 NILSSON[1] described the structure of ceroptene, an enolic constituent of the waxy exudate of *Pityrogramma triangularis* leaves. Other work from his laboratory[2,3] dealt with the structure of several chalcones and dihydrochalcones in *P. chrysophylla* varieties. Preliminary studies in this laboratory were recently undertaken with a view to the possible biochemical relationships of enolic and phenolic compounds present in the foliar exudate of *P. calomelanos*. This paper describes the isolation, structural analysis, and chemical origin of one of these compounds.

RESULTS AND DISCUSSION

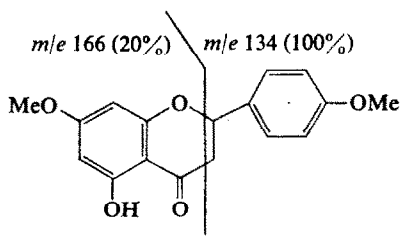
The initial approach to the problem involved a duplication of Nilsson's procedure[1] for isolation of ceroptene. The sodium hydroxide soluble fraction of *Pityrogramma calomelanos* exudate yielded two major bands on thin-layer chromatography (TLC) using silica gel. The slower band (R_f 0·27) was yellow–orange and may contain ceroptene or ceroptene-like molecules. The second band (R_f 0·51) had a striking cream–white fluorescence under u.v. light (3660 Å). Elution of the slower yellow–orange band with acetone yielded a mixture of substances as judged by the wide melting range. Elution of the faster band with acetone yielded a compound which crystallized easily from acetone in long, colorless needles. The m.p., 116·5–117·5°, was sufficiently close to the value reported (119–120°) by Nilsson[3] for 4′,7-dimethoxy-5-hydroxyflavanone to warrant further comparisons with the flavanone earlier isolated from *P. chrysophylla* var. *heyderi*.

[1] M. NILSSON, *Acta Chem. Scand.* **13**, 750 (1959).
[2] M. NILSSON, *Acta Chem. Scand.* **15**, 154 (1961).
[3] M. NILSSON, *Acta Chem. Scand.* **15**, 211 (1961).

Ferric chloride gave a mauve color with the compound, a color which is considered[4] characteristic for 5-hydroxyflavanones. A pale orange was observed with diazotized *p*-nitroaniline indicating the presence of at least one phenolic group. An intense blue color developed on mixing a few crystals of the compound with a drop of concentrated nitric acid, a reaction described by Marini-Bettolo[5] as characteristic of naringen-4',7-dimethyl ether. The u.v. spectrum showed a band at 288 nm and a shoulder at about 330 nm. No displacement of the 288 nm band resulted upon addition of sodium acetate which indicates the absence of an ionizable phenolic function at C-7.[6] The i.r. spectrum disclosed the presence of a hydrogen bonded carbonyl function (1645 cm^{-1}) within the range observed by Wagner[7] for 5-hydroxyflavonoids and also of aromatic ether, phenolic OH, methylene, and a complex aromatic substitution pattern.

Mass spectral analysis of the compound gave a value of 300 for the molecular weight which agrees with the molecular formula $C_{17}H_{16}O_5$. The NMR spectrum, when integrated, agreed with the presence of sixteen protons in the molecule. Absorption in the region 2·5 to 4·0τ showed the presence of six aromatic protons. The two doublets ($J=10$ c/s) centered at 2·62τ and 3·05τ represent the protons of a 4'-substituted flavonoid B-ring while the slightly split ($J=2$–3 c/s) quartet at 3·96τ represents the six and eight protons on the A-ring. The peak at 6·20τ (six protons) confirms the presence of two methyl ether groups. The nature of the molecule is firmly established by the presence of a quartet at 4·65τ (one proton) ($J_{cis}=4$ c/s, $J_{trans}=12$ c/s) and two overlapping quartets (two protons) centered at 7·04τ which represent, respectively, the protons at positions 2 and 3 of a flavanone. These assignments are in excellent accord with those made by Mabry and co-workers[8] on a series of known flavanones.

The mass spectral fragmentation pattern of the molecule agrees with the structure 4',7-dimethoxy-5-hydroxyflavanone. The structure is shown below with a plausible scission indicated. The numbers in parentheses represent fragment abundance values. Further breakdown of the right-hand fragment into the *p*-methoxybenzyl ion would explain the peak



at *m/e* 121 (62 per cent). Elimination of carbon monoxide from the left-hand fragment would account for the *m/e* 138 peak (16 per cent).

It is well known that an equilibrium exists between a flavanone and the corresponding chalcone and that chalcones can easily be cyclized by the action of alkali. This being the case, it immediately became apparent that the sodium hydroxide (0·5 M) extraction step in Nilsson's procedure[1] should be sufficient to effect conversion of a significant quantity of

[4] F. M. DEAN, *Naturally Occurring Oxygen Ring Compounds*, p. 335. Butterworths, London (1963).
[5] G. B. MARINI-BETTOLO and M. R. FALCO, *Ann. Chim.* **41**, 221 (1951).
[6] J. B. HARBORNE, *Methods in Polyphenol Chemistry*, p. 26. Pergamon Press, Oxford (1964).
[7] H. WAGNER, *Methods in Polyphenol Chemistry*, pp. 40–41. Pergamon Press, Oxford (1964).
[8] T. J. MABRY, J. KAGAN and H. RÖSLER, *Nuclear Magnetic Resonance Analysis of Flavonoids*. The University of Texas Publication, Austin (1964).

naturally existing chalcone to the flavanone. In order to examine this possibility, plant material was extracted with ether and the extract was subjected immediately to TLC analysis. Chromatograms run on this initial extract showed total absence of the flavanone. Extraction of the crude preparation with 0·5 M sodium hydroxide solution yielded a yellow–orange solution. After acidification the mixture was extracted with ether several times. Evaporation of the combined ether extracts yielded a yellow solid. Chromatography of this material showed a strong band corresponding to the running position of the flavanone originally isolated. These results are interpreted to mean that 2′,6′-dihydroxy-4,4′-dimethoxychalcone is a naturally occurring constituent of the exudate of *P. calomelanos* leaves and that this compound is converted in large measure into the flavanone during isolation. This seems to be a reasonable conclusion on chemosystematic grounds since Nilssons found the same chalcone in *P. chrysophylla* var. *heyderi*.[3] This particular substitution pattern may represent a useful systematic character in this genus and is an aspect of the chemistry of this interesting group of plants that might prove fruitful for future investigation.

EXPERIMENTAL

Source of Plant Material

The *Pityrogramma calomelanos* (L.) Link plants used for this study were grown in the departmental greenhouses and were derived from plants collected and identified by Dr. T. M. C. Taylor in Hawaii. Living material will be maintained in the departmental collection.

Extraction of the Plant Material

The procedure was a slightly modified version of the procedure described by Nilsson.[1] Leaves were washed with ether and the solids obtained upon evaporation of the solvent were dissolved in dimethylformamide. The dimethylformamide solution was repeatedly extracted with petrol. ether (60–120°), and then poured into water. The precipitated yellow solids were extracted with ether. The ether solution was immediately extracted, first with saturated Na_2CO_3, then with 0·5 M NaOH. Acidification of the extracts with HCl in ice afforded crude solid precipitates. The sodium carbonate soluble material was not examined further in this work.

Thin-Layer Chromatography

TLC analyses were run on 0·5 mm thick layers of Silica Gel G (Stahl) on 200 × 200 mm glass plates. The plates were activated for 1 hr at 120° and were banded as soon as they had cooled to room temperature. All plates were run in reagent grade CH_2Cl_2 and bands were located in u.v. light of wavelength 3660 Å.

Instrumentation

The i.r. spectrum of the compound was run as a KBr disc using a Unicam SP-200G instrument. The u.v. absorption was determined using a Unicam SP-800 instrument. A Varian HA-100 instrument was used for determining the NMR spectrum. Tetramethylsilane was employed as the standard and deuterochloroform was used as the solvent. Mass spectral analysis was performed by the Morgan Schaffer Corporation, Montreal.

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