Early fractions contained waxy materials which were not characterized. Eluates with $CHCl_3$–EtOAc (9:1 to 6:1) yielded crystalline residues which proved to be vulgarin. Recrystallized from CH_2Cl_2–ether, it melted at 174–175°, and was identical (m.p., i.r., NMR) with an authentic specimen of vulgarin. The yield was 3·2 g.

TLC both of the original extracts and of various column fractions, using authentic artemorin, anhydroverlotorin and verlotorin for comparison, showed that none of these compounds was present. Polar materials in later column fractions were rechromatographed but no further crystalline compounds were obtained.

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CYTOTOXIC FLAVONOLS FROM *BACCHARIS SAROTHROIDES**

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Abstract—Two cytotoxic flavonols, isolated from the leaves and twigs of *Baccharis sarothroides*, have been characterized as 3,4′-dimethoxy-3′,5,7-trihydroxyflavone and centaureidin.

INTRODUCTION

IN THE course of a continuing search for tumor inhibitors from plant origin, we found that alcoholic extracts of *Baccharis sarothroides A. Gray* (Compositae)† showed significant inhibitory activity against cells derived from human carcinoma of the nasopharynx carried in cell culture (KB).[1] We report herein the isolation and identification of cytotoxic flavonols, 3,4′-dimethoxy-3′,5,7-trihydroxyflavone (I) and centaureidin (IV)‡ from *B. sarothroides*. This appears to be the first report of the characterization of specific flavonoids from a *Baccharis* species, although flavonoids have been noted to occur in *Baccharis rosmarinifolia*.[2] In an earlier study, we have isolated two cytotoxic flavonols, eupatin and eupatoretin from *Eupatorium semiserratum* (Compositae).[3] It is noteworthy that centaureidin possesses the same oxygenation pattern as eupatin and eupatoretin.

* Part LXIII in the series "Tumor inhibitors". For part LXII, see S. M. KUPCHAN, M TAKASUGI, R. M. SMITH and P. S. STEYN, *J. Org. Chem.* in press.

† Twigs and leaves of *Baccharis sarothroides* were collected in California in May 1967. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr , U S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with U.S.D.A. by the Cancer Chemotherapy National Service Center (C C.N S.C.).

‡ 3,4′-Dimethoxy-3′,5,7-trihydroxyflavone (I) and centaureidin (IV) showed cytoxicity (ED_{50}) against KB cell culture at 2 4 μg/ml and 2·5 μg/ml, respectively.

[1] Cytotoxicity was assayed under the auspices of the C.C.N.S.C. and the procedures were those described in *Cancer Chemotherapy Rept.* **25**, 1 (1962).

[2] E. P. BENIMELI, *Anales Fac. Quim. Farm. Univ. Chile* **16**, 133 (1964).

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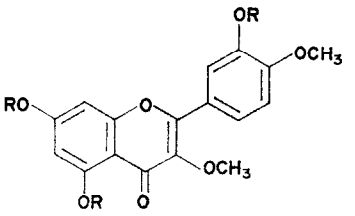
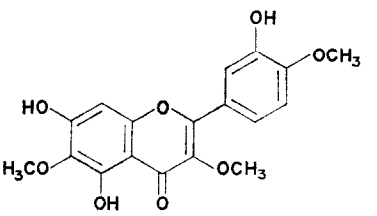
RESULTS AND DISCUSSION

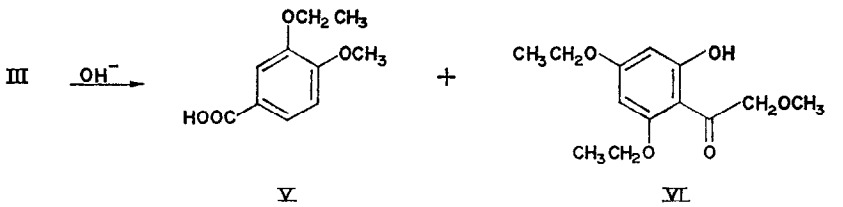
Elemental analysis of I supported the empirical formula $C_{17}H_{14}O_7$. The u.v. spectrum showed a peak at 357 nm, indicative that it is a flavonol rather than a flavone. The absorption at 257 and 269 nm established a 3′,4′-substitution pattern. The NMR spectrum of I showed the presence of two methoxy groups. Methylation afforded pentamethylquercetin (II) which established a 3,3′,4′,5,7-oxygenation pattern. The location of the three remaining hydroxyl groups of I was established by preparing the triethyl ether (III), which was subjected to mild alkaline degradation. The acidic product was identified by its NMR spectrum and melting point as 3-ethoxy-4-methoxybenzoic acid (V). The neutral fraction afforded an



I, R=H
II, R=CH_3
III, R=CH_3CH_2



IV Centaureidin



V VI

acetophenone which was identified by its melting point, NMR spectrum, and analysis as 4,6-diethoxy-2-hydroxy-ω-methoxy-acetophenone (VI). These degradative experiments firmly established the flavonol to be 3,4′-dimethoxy-3′,5,7-trihydroxyflavone (I). This appears to be the first isolation of this flavonol as a natural product.[4]

Centaureidin (IV) was identified by comparison of its physical properties with those reported in the literature. The compound had previously been isolated as a glycoside[5] and as a free flavone.[6]

EXPERIMENTAL

M.ps were determined with a Thomas–Hoover Unimelt apparatus and are corrected. Analyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Isolation of Flavonols

Coarsely ground leaves and twigs (1·12 kg) of *B. sarothroides* were extracted with C_2H_5OH to yield 380 g of material which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was evaporated to dryness and

[4] The synthesis of 3,4′-dimethoxy-3′,5,7-trihydroxyflavone has been reported: F. E. KING, T. J. KING, K. SELLARS, *J. Chem. Soc.* 92 (1952).
[5] L. FARKAS, L. HÖRHAMMER and H. WAGNER, *Tetrahedron Letters* 727 (1963); 382 (1964).
[6] F. BOHLMANN and C. ZDERO, *Tetrahedron Letters* 3239 (1967).

the residue was partitioned between Skellysolve B and 10% aq. CH_3OH to yield 80 g of CH_3OH-soluble material. Chromatography on a silica gel column by elution with $CHCl_3$ and increasing percentages of CH_3OH afforded an active fraction which upon rechromatography on SilicAR CC-7 with $CHCl_3$–CH_3OH afforded two partially crystalline fractions. Recrystallization of each from methanol yielded yellow crystals of centaureidin (IV, 0·12 g), m.p. 195° (lit. 197°)[7] and 3,4′-dimethoxy-3′,5,7-trihydroxyflavone (I, 0·18 g), m.p. 235–6° (lit. 232–3°);[4] u.v. max (95% C_2H_5OH) 257 (ε 17,500), 269 (ε 15,500), 357 nm (ε 15,200); i.r. (KBr) 3·01, 3·40, 6·08, 6·2, 6·3 μ; NMR (d_6-DMSO) τ −2·75 (1H, s), 2·40 (2H, m), 2·86 (1H, J = 8·5 Hz), 3·53 (1H, d, J = 1 Hz), 3·76 (1H, d, J = 1 Hz), 6 09 (1H, s), 6·15 (1H, s). (Found: C, 61·69; H, 4 33. Calc. for $C_{17}H_{14}O_7$: C, 61·82; H, 4·27%)

3,3′,4′,5,7-*Pentamethoxyflavone* (*II*)

A solution of 3,4′-dimethoxy-3′,5,7-trihydroxyflavone (I, 50 mg) in dry acetone (10 ml) was refluxed for 18 hr with anhydrous K_2CO_3 (3 g) and $(CH_3)_2SO_4$ (1 ml). After cooling, the solution was filtered, the solvent removed *in vacuo*, and the residue dissolved in $CHCl_3$. The $CHCl_3$ solution was washed twice with saturated NaCl solution, dried ($MgSO_4$), and the solvent removed by evaporation. The crystalline residue was recrystallized from C_2H_5OH to yield 39 mg of light yellow needles, m p. 147–8° (lit. 147–8°,[8] 152–2°);[9] i.r. (KBr) 3·40, 6·15–6·20, 6·9 μ; u.v. max (95% C_2H_5OH) 250 (ε 22,600), 265 (ε 17,400), 341 nm (ε 22,000); (Found: C, 64·46; H, 5 44. Calc. for $C_{20}H_{20}O_7$: C, 64·51; H, 5 41%)

3,4′-*Dimethoxy*-3′,5,7-*trihydroxyflavone* (*III*)

To a solution of 3,4′-dimethoxy-3′,5,7-trihydroxyflavone (I, 150 mg) in dry acetone (15 ml) were added dry K_2CO_3 (3 g) and C_2H_5I (3 g) and the mixture refluxed for 20 hr. After cooling, the solution was filtered, and then the acetone evaporated. The residue was dissoved in $CHCl_3$ which was washed twice with saturated NaCl solution and dried ($MgSO_4$). The solvent was evaporated to yield 170 mg. Recrystallization from C_2H_5OH afforded 87 mg of colorless crystals, m.p. 108–9°, i.r. (KBr) 3·40, 6·18–6·25, 7·00 μ; u.v. max (95% C_2H_5OH) 250 (ε 28,700), 266 (ε 21,200), 341 nm (ε 26,000); NMR ($CDCl_3$) τ 2·27 (2H, m), 2·97 (1H, d, J = 8·5 Hz), 3·48 (1H, d, J = 1 Hz), 3·62 (1H, d, J = 1 Hz), 5·66–5 96 (6H, m), 6 03 (3H, s), 6·10 (3H, s), 8·45 (3H, t), 8 50 (3H, t), 8·55 (3H, t). (Found: C, 66·47; H, 6·13. Calc. for $C_{23}H_{26}O_7$: C, 66·65; H, 6·32%.)

Alkaline Degradation of 3,4′-*Dimethoxy*-3′,5,7-*triethoxyflavone* (*III*)

A solution of the triethyl ether III (30 mg) in 50% KOH (13 ml) and C_2H_5OH (6·5 ml) was refluxed under N_2 for 16 hr. The reaction mixture was cooled and acidified with 20% H_2SO_4, then extracted several times with ether. The ethereal extract was washed with five 50-ml portions of 5% $NaHCO_3$, dried ($MgSO_4$), and evaporated to dryness to afford crystalline acetophenone VI. It was recrystallized to yield 53 mg of VI, m p. 109–10° (lit. 110–1°,[10] 111–2°[11]); i.r. (KBr) 2·92, 3·40, 6·19, 6·26 μ; u.v. max (95% C_2H_5OH) 290 nm (ε 19,200); NMR ($CDCl_3$) τ −3·75 (1H, s), 3·90 (1H, d, J = 1 Hz), 4·10 (1H, d, J = 1 Hz), 5·35 (2H, s), 5·91 (2H, q, J = 7·5 Hz), 5·93 (2H, q, J = 7·5 Hz), 6·50 (3H, s), 8·50 (3H, t, J = 7·5 Hz), 8·60 (3H, t, J = 7·5 Hz). (Found: C, 61·48; H, 7·19. Calc. for $C_{13}H_{18}O_5$: C, 61·40; H, 7·14%.)

The bicarbonate extract was acidified with dilute HCl and extracted with ether; the ethereal solution was dried ($MgSO_4$) and evaporated to afford a colorless solid. Recrystallization from water gave 3-ethoxy-4-methoxybenzoic acid (V) as colorless needles, m.p. 163–4° The mixed m.p. with authentic material showed no depression.

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