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FLAVONOID CONSTITUENTS OF *HYMENOXYS SCAPOSA* DC. (COMPOSITAE)

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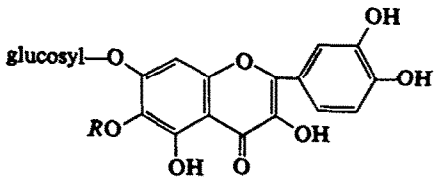
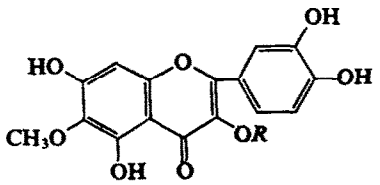
Abstract—Four flavonol glycosides, quercetagitrin, patulitrin, patuletin 3-*O*-rutinoside, and patuletin 3-*O*-glucoside, were isolated from *Hymenoxys scaposa*. The latter two compounds are reported here for the first time.

INTRODUCTION

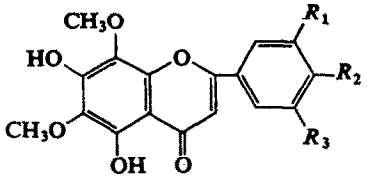
IN CONNEXION with a general chemotaxonomic investigation of *Hymenoxys* (Compositae), we report here the major flavonoids in *H. scaposa*, a species known to produce different flavonoids according to its geographical origin.[1] Two well-known[2] flavonol glycosides, patulitrin (I) and quercetagitrin (II), were found to be typical of the flower heads of plants from Texas, and two new glycosides of patuletin, the 3-*O*-rutinoside (III) and 3-*O*-glucoside (IV), are characteristic for flower heads from plants collected in Utah, Colorado, Wyoming and north-eastern Arizona. The latter two compounds also occur widely in leaves of plants from other areas. Three flavones, scaposin (V), demethoxysudachitin (VI), and hymenoxin (VII), whose isolation and structure determinations have already been reported,[3,4] are common in leaves of plants from all populations of the species.



(I) $R = CH_3$
(II) $R = H$



(III) R = rutinosyl
(IV) R = glucosyl



(V) $R_1 = R_2 = OCH_3$; $R_3 = OH$
(VI) $R_1 = R_3 = H$; $R_2 = OH$
(VII) $R_1 = R_2 = OCH_3$; $R_3 = H$

[1] P. SEELIGMANN and R. E. ALSTON, *Brittonia* **19**, 205 (1967).
[2] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965); *Comparative Biochemistry of the Flavonoids*, Academic Press, London and New York (1967).
[3] M. B. THOMAS and T. J. MABRY, *J. Org. Chem.* **32**, 3254 (1967).
[4] M. B. THOMAS and T. J. MABRY, *Tetrahedron*, in press (1968).

RESULTS

Isolation and Identification of Patulitrin (I) and Quercetagitrin (II)

Polyamide chromatography of the crude methanolic extract of the flower heads of *Hymenoxys scaposa* collected near Austin, Texas, afforded patulitrin (I), pale yellow prisms from aqueous MeOH, m.p. 254–256°, and quercetagitrin (II), an amorphous solid from aqueous MeOH, m.p. 225–230°. PMR analysis of the trimethylsilyl ether derivative of I in CCl_4 showed a pattern typical for a flavonoid having 3′,4′-oxygenation in ring B. One other flavone nucleus proton gave a singlet at 6·65 ppm (δ), which could be attributed to H-8, H-6 or H-3. The trimethylsilyl ether derivative was exposed to the atmosphere for 15 min (conditions known to selectively detrimethylsilylate the C-5 position) and the PMR analysis was repeated. The latter spectrum showed a new peak at 12·35 ppm (C-5 hydrogen-bonded hydroxyl group). In addition, the singlet at 6·65 ppm had moved upfield to 6·52 ppm, a behavior characteristic[5] of C-8 protons in flavonoids. Compound I was, therefore, substituted at C-3, -3′, -4′, -5, -6 and -7. The PMR spectrum of the fully trimethylsilylated compound also indicated the presence of a methoxyl group (singlet, 3·84 ppm) and a sugar residue (complex multiplets near 5·0 and 3·6 ppm). The u.v. spectrum of compound I showed maxima at 258 and 375 nm. The presence of an *ortho*-dihydroxy group was indicated by the bathochromic shift of band I (15 nm) in NaOAc–H_3BO_3.[6] Furthermore, a band I shift of 81 nm in the presence of $AlCl_3$ and 56 nm with $AlCl_3$–HCl showed that the OH groups at C-3, C-3′ and C-4′ were free.[7] The flavonoid therefore had OH groups at C-3, -3′, -4′ and -5 with a sugar residue and a OMe group occupying C-6 and C-7. Methylation of the glycoside with dimethyl sulphate followed by acid hydrolysis gave 7-hydroxy-3,3′,4′,5,6-pentamethoxyflavone, m.p. 232–234°, identical in all respects with an authentic sample.[8] Further methylation of the latter compound gave hexamethoxyquercetagetin, m.p. and m.m.p. 141–142°, with an authentic sample.[8] The sugar moiety was shown to be glucose by GLC analysis,[9] and by hydrolysis with β-glucosidase. In addition to glucose, hydrolysis also gave patuletin, m.p. 260–261°. The flavonol must therefore be patulitrin[10] (I).

The second flavonol glycoside from the Texas populations of *H. scaposa* was unusual in appearing as a dull black spot on paper chromatograms under u.v. light. The aglycone obtained on acid hydrolysis exhibited the same property. This color characteristic had previously been observed for quercetagitrin (II) and its aglycone quercetagetin.[2] The *H. scaposa* glycoside was shown to be quercetagitrin[11] by u.v. and PMR (of the trimethylsilyl ether) analysis and by the GLC determination[9] of the trimethylsilyl ether of the glucose obtained on acid hydrolysis. Methylation of the aglycone using dimethyl sulphate afforded hexamethoxyquercetagetin, m.p. 141–142°, a result which confirmed the oxygenation pattern shown in II.

[5] T. J. MABRY, J. KAGAN and H. RÖSLER, *Nuclear Magnetic Resonance Analysis of Flavonoids*. The University of Texas Publication No. 6418, Austin, Texas (1964).
[6] L. JURD, "Spectral properties of flavonoid compounds", in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), pp. 107–155. Macmillan, New York (1962).
[7] Details for the application of the u.v. spectral shifts observed in the presence of $AlCl_3$ and $AlCl_3$–HCl for detecting *ortho*-dihydroxy groups in flavonoids will be reported elsewhere (K. R. MARKHAM and T. J. MABRY, *Phytochem.*, in press (1968).
[8] Supplied by Professor H. Wagner, Institut für Pharmazeutische Arzneimittellehre der Universität München.
[9] J. KAGAN and T. J. MABRY, *Anal. Chem.* 37, 289 (1965).
[10] N. R. BANNERJEE and T. R. SESHADRI, *Proc. Indian Acad. Sci.* 44, 284 (1956).
[11] P. S. RAO and T. R. SESHADRI, *Proc. Indian Acad. Sci.* 14, 298 (1941).

Isolation and Structure Determination of Patuletin 3-O-*Rhamnosylglucoside* (*III*) *and* 3-O-*Glucoside* (*IV*)

Polyamide column chromatography of the residue obtained from a crude methanolic extract of the leaves of *H. scaposa* collected near Austin, Texas, afforded a new flavonol glycoside (III). Compound III, m.p. 175–180°, pale yellow needles from aqueous methanol, was hydrolyzed with acid to patuletin, identical in all respects with the material obtained from the hydrolysis of patulitrin. The sugars obtained on hydrolysis of the glycoside were identified as rhamnose and glucose by the GLC analysis[9] of their trimethylsilyl ethers. The PMR spectrum of the trimethylsilyl ether of the glycoside showed that the rhamnose and glucose were present as a C-3 *O*-rutinosyl group.[12] The u.v. spectral data (37 nm shift of band I in $AlCl_3$–HCl) supported the assignment of the glycoside moiety to C-3 as did the dark fluorescence of the glycoside when examined on paper under u.v. light. Complete methylation of compound III followed by acid hydrolysis yielded a compound, m.p. 190–191°, which exhibited a band I shift in the presence of $AlCl_3$–HCl of 63 nm, typical for a free C-3 OH. The glycoside is, therefore, patuletin 3-*O*-rutinoside (III).

Column chromatography of the crude aqueous methanolic (1:1) extract of flower heads of *H. scaposa* collected in Arizona[13] yielded pale yellow needles of a second new flavonol glycoside (IV), m.p. 237–238°. Acid hydrolysis of compound IV gave patuletin. The u.v. spectra of compound IV in MeOH and in MeOH with diagnostic reagents[6] were almost identical with those obtained for patuletin 3-*O*-rutinoside (III). PMR analysis of the trimethylsilyl ether of the glycoside and the GLC determination of the trimethylsilyl ether of the sugar obtained on β-glucosidase hydrolysis of IV showed that the sugar moiety was glucose. The glucoside is, therefore, patuletin 3-*O*-β-D-glucoside (IV).

EXPERIMENTAL

Melting points are uncorrected. U.v. spectra were determined in absolute methanol. PMR spectra were determined in CCl_4 with tetramethylsilane as internal reference. All hydrolyses and methylations were carried out by standard procedures.

Isolation of Patulitrin (*I*) *and Quercetagitrin* (*II*)

Dried, ground flower heads of *Hymenoxys scaposa* DC.[14] (98 g) collected approximately 25 miles northwest of Austin, Texas, 23 April, 1966, were extracted first with pet. ether (3 × 1 l., 24 hr each), then with CH_2Cl_2 (3 × 1 l., 24 hr each) and finally with MeOH (3 × 1 l., 24 hr each). The methanolic extracts were combined and evaporated to dryness. The residue (about 15 g) was dissolved in hot water and half the solution was chromatographed on a polyamide column (25 cm × 4·5 cm); 20 ml fractions were collected. The column was eluted first with water (fractions 1–22) then H_2O:MeOH (4:1; fractions 23–35) and finally aqueous MeOH (1:1; fractions 36–122). Fractions 57–67 afforded yellow prisms of patulitrin (59 mg), m.p. 254–256° after recrystallization from aqueous MeOH (lit.[8] m.p. 253–254°). The compound had λ_{max} 258 and 375 nm; λ_{max} ($AlCl_3$) 276, 346 (infl.) and 456 nm; λ_{max} ($AlCl_3$–HCl) 270 and 431 nm; λ_{max} (NaOAc) 258 and 382 nm; λ_{max} (NaOAc and H_3BO_3) 262 and 390 nm; PMR of trimethylsilyl ether: 7·57–7·83 (multiplet, H-2′ and H-6′); 6·83 (doublet, J = 9 c/s, H-5′); 6·65 (singlet, H-8); 4·84–5·11 (multiplet, glucosyl C-1 proton); 3·84 (singlet, C-6 OMe); and 3·33–3·80 ppm (multiplet, 6 glucosyl protons).

Fractions 88–105 yielded quercetagitrin (11 mg) as an amorphous yellow solid from aqueous methanol. Quercetagitrin had λ_{max} 260, 273 (infl.) and 363 nm; λ_{max} ($AlCl_3$–HCl) 271 and 414 nm; λ_{max} (NaOAc) 259, 279 and 376 nm; PMR of the trimethylsilyl ether: near 7·58 (multiplet, H-2′, H-6′); 6·63 (doublet, J = 9 c/s, H-5′); 6·50 (singlet, H-8); near 5·1 (multiplet, glucosyl C-1 proton); 3·3–3·8 (multiplet, 6 glucosyl protons).

[12] H. RÖSLER, T. J. MABRY, M. F. CRANMER and J. KAGAN, *J. Org. Chem.* **30**, 4346 (1965).
[13] Collected 4 miles south of Sedona, Arizona, on U.S. Highway 179, on 18 May, 1966, by the late R. E. Alston.
[14] Voucher No. 255332, The University of Texas Herbarium, Austin, Texas.

Patuletin 3-O-*Rutinoside* (*III*)

Dried, ground leaves of *H. scaposa*[14] (196 g, from the same collection as in the above experiment) were extracted as previously described. The residue from the crude methanolic extract was dissolved in aqueous MeOH (1:1) and chromatographed on polyamide. The column was eluted with aqueous MeOH (1:1) and 20 ml fractions were collected. Evaporation of fractions 37–48 yielded material which crystallized from aqueous MeOH as pale yellow needles (115 mg), m.p. 175–180°. The compound, patuletin 3-rutinoside, had λ_{max} 353 (ϵ 16,900), 267 infl. (ϵ 16,300) and 258 (ϵ 17,100) nm; λ_{max} (NaOMe) 272 and 411 nm; λ_{max} ($AlCl_3$–HCl) 276 and 390 nm; λ_{max} (NaOAc) 272 and 390 nm; and λ_{max} (NaOAc–H_3BO_3) 266 and 382 nm; PMR of trimethylsilyl ether: 7·31–7·52 (multiplet, H-2′ and H-6′); 6·84 (doublet, J=9 c/s, H-5′); 6·49 (singlet, H-8); 5·93 (multiplet, glucosyl C-1 proton); 4·78 (doublet, J=2 c/s, rhamnosyl C-1 proton); 3·72 (singlet, C-6 OMe); 3·20–3·80 (multiplet, 10 rhamnosylglucosyl protons); and 0·7–0·8 ppm (multiplet, rhamnosyl CH_3).

Patuletin 3-O-*Glucoside* (*IV*)

Dried ground flower heads of *H. scaposa* (45 g) collected in Arizona[13] were soxhlet extracted with pet. ether (250 ml, 24 hr) and then with CH_2Cl_2 (250 ml, 24 hr). The flowers were then extracted with cold aqueous MeOH (1:1; 2 × 750 ml, 24 hr each). The residue from the crude methanolic extract was chromatographed on polyamide as described above using H_2O:MeOH (4:1) as eluent. Fractions of 100 ml were collected. Evaporation of fractions 12 and 13 gave a residue, which crystallized from aqueous MeOH (1:1) as very pale yellow needles (11 mg), m.p. 237–238°. The compound, patuletin 3-*O*-glucoside, had λ_{max} 354 (ϵ 18,150), 267 infl. (ϵ 17,000), and 259 (ϵ 18,300) nm; λ_{max} (NaOMe) 273, 337 and 412 nm; λ_{max} (NaOAc) 273 and 382 nm; λ_{max} ($AlCl_3$–HCl) 271 and 383 nm; PMR of the trimethylsilyl ether: 7·67–7·37 (multiplet, H-2′ and H-6′); 6·81 (doublet, J=9 c/s, H-5′); 6·44 (singlet, H-8); 5·85 (multiplet, glucosyl C-1 proton); 3·69 (singlet C-6 OMe); and 3·82–3·8 ppm (multiplet, 6 glucosyl protons).

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