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FLAVONOIDS FROM THE LEAF RESIN OF *ADENOSTOMA SPARSIFOLIUM**

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Key Word Index—*Adenostoma sparsifolium*; Rosaceae; flavonoids; 3, 7-dihydroxy-5, 6-dimethoxyflavone; 3, 5, 7-trihydroxy-8-methoxyflavone; galangin; pinocembrin.

Abstract—Four flavonoids were identified from the external leaf extract of *Adenostoma sparsifolium*: the two new flavones 3, 7-dihydroxy-5, 6-dimethoxyflavone and 3, 5, 7-trihydroxy-8-methoxyflavone and the known compounds galangin and pinocembrin.

Adenostoma sparsifolium (Torr.) (Rosaceae) is a shrub or small tree that is dominant in the Chaparral areas of California and Baja California, Mexico [1]. Its leaves are covered by a thick layer of lipophilic resin which represents 10–20% dry wt and consists mainly of phenolic material. The major flavonoids are characterized by an unsubstituted B-ring. Besides pinocembrin and galangin, we isolated and identified the new flavones 3, 7-dihydroxy-5, 6-dimethoxyflavone (**1**) and 3, 5, 7-trihydroxy-8-methoxyflavone (**5**).

1 exhibited a yellow-green fluorescent colour under UV which did not change after fuming with ammonia, thus indicating a flavone with a free 3-hydroxyl group and the 5-hydroxyl group either lacking or substituted [2]. The MW of 314 suggested the presence of two hydroxyls and two methoxyl groups [3]. A strong peak at *m/z* 105 in the mass spectrum was characteristic for an unsubstituted B-ring. In the sodium acetate spectrum a bathochromic shift of 10 nm indicated a free 7-hydroxyl group while the second hydroxyl was assigned to the C-3 position. The C-5 position is substituted by one of the methoxyl groups, since the ^{1}H NMR spectrum showed no singlet around 8 ppm, typical for the deshielded proton at C-5 [4]. The remaining methoxyl group could be located either at the C-6 or C-8 position. Several experiments confirmed the location of the methoxyl group at C-6. Thus, the ^{1}H NMR spectrum of the permethylated compound **3** showed a singlet at 6.75 ppm typical for the H-8 proton of a flavone with methoxyl groups at 5, 6, 7 [5]. A Nuclear Overhauser Effect (NOE) was not observed for **1** after irradiation of the two methoxyl groups at 4.02 and 4.05 ppm or the singlet at 6.82 ppm, respectively [6]. Selective demethylation of **3** yielded 5, 7-dihydroxy-3, 6-dimethoxyflavone **4** which was identified from its UV spectrum [7], the $AlCl_3$–HCl spectrum exhibiting a shift of 25 nm, typical for a 6-methoxyl group. Thus **1** was identified as 3, 7-dihydroxy-5, 6-dimethoxyflavone. The other new compound, 3, 5, 7-trihydroxy-8-methoxyflavone (**5**), had a MW of 300 which indicated the presence of three hydroxyl groups and one methoxyl group [3]. It was also characterized by an unsubstituted B-ring as indicated by ^{1}H NMR and mass spectra. The hydroxylation pattern at positions C-3, C-5, and C-7 was evident from UV spectroscopy, especially the shifts induced with NaOMe and $AlCl_3$–HCl. A strong peak of almost 100% at *m/z* 285 indicated the location of the methoxyl group at either C-6 or C-8. Differentiation between these two positions was achieved by methylation of **5** to give 5-hydroxy-3, 7, 8-trimethoxyflavone identified by UV and ^{1}H NMR spectra and by comparison with literature data for 5-hydroxy-3, 6, 7-trimethoxyflavone [8] and 5-hydroxy-3, 7, 8-trimethoxyflavone [9]. The presence of the 8-methoxy group was established by the $AlCl_3$–HCl test which induced a shift of 53 nm. The proton singlet at 6.4 ppm was assigned to the H-6 proton [9]. The structure of **5** is therefore 3, 5, 7-trihydroxy-8-methoxyflavone. Although a synthesis of this flavone has already been published [10], this is to our knowledge the first report of its natural occurrence.

EXPERIMENTAL

Plant material was collected in May 1981 near Oak Grove in San Diego County, CA. A voucher specimen is deposited in the UCI Herbarium No. 18851.

The resinous leafy branches were extracted with $CHCl_3$ for 3 min. The $CHCl_3$ was evapd and the residue redissolved in MeOH and applied to a Sephadex LH-20 column for preliminary separation using MeOH as eluting system. Fractions 1, 2 and 4 contained **1**, pinocembrin and galangin in almost pure amounts. Fraction 3 contained a mixture of **5** and another flavonoid which has yet to be identified. **5** was

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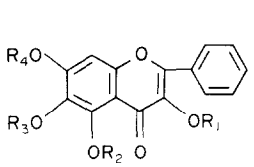

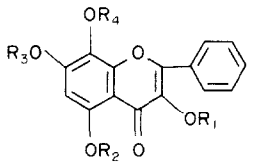

1 $R_1 = R_4 = H$; $R_2 = R_3 = Me$
2 $R_1 = R_4 = Ac$; $R_2 = R_3 = Me$
3 $R_1 = R_2 = R_3 = R_4 = Me$
4 $R_1 = R_3 = Me$; $R_2 = R_4 = H$

5 $R_1 = R_2 = R_3 = H$; $R_4 = Me$
6 $R_1 = R_3 = R_4 = Me$; $R_2 = H$

isolated by prep. TLC on polyamide in the solvent system C_6H_6–MeCOEt–MeOH (30:13:7). Final purification of all isolated compounds was achieved by CC on Sephadex LH-20 with MeOH as solvent. The amount of phenolic and acidic material (93% of total resin) was determined by partitioning of the resin between $CHCl_3$ and 5% aq. NaOH. Methylation of **1** with diazomethane in MeOH for 48 hr at room temp. yielded **3**, methylation of **5** yielded **6**; the methylated derivatives were purified by column chromatography on Sephadex LH-20 with MeOH as solvent.

Demethylation of **3** was achieved as described in ref. [11]. The resulting $CHCl_3$ fraction was chromatographed by Si gel TLC in $CHCl_3$. The main compound (**4**), was visible as a deep purple band under UV and was extracted with MeOH and purified on Sephadex LH-20 eluted with MeOH. Acetylation of **1** was performed in the usual manner. The acetylated compound (**2**) was purified by Si gel TLC (solvent $CHCl_3$) and chromatography on a Sephadex LH-20 column (solvent MeOH).

3,7-*Dihydroxy*-5, 6-*dimethoxyflavone* (**1**). R_f values on Whatman paper MM3: 0.22 (15% HOAc), 0.85 (TBA). UV λ_{max}^{MeOH} nm: 262; 290 sh; 328; NaOMe: 274; 373; $AlCl_3$: 270; 314; 340 sh; 406; $AlCl_3$–HCl: 270; 314; 340 sh; 406; NaOAc: 273; 373; H_3BO_3: 283; 328; 426; ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ 4.02 (3H, *s*, C-6 OMe), 4.05 (3H, *s*, C-5 OMe), 6.82 (1H, *s*, C-8), 7.45 (3H, *m*, C-3′, 4′, 5′), 8.17 (2H, *m*, C-2′, 6′). (C_6D_6) δ 3.7 (3H, *s*, C-5 OMe), 3.8 (3H, *s*, C-6 OMe). EIMS (70 eV, probe) *m/z* (rel. int.) 314 (M^+, 45), 299 ($M^+ - CH_3$, 100), 283($M^+ - OCH_3$, 7), 271($M^+ - CH_3CO$, 10), 179 ($A_1 + H - CO$, 10), 167($A_1 + H - 32$, 12), 105(B_2, 45).

5, 6-*Dimethoxy*-3, 7-*diacetoxyflavone* (**2**). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ 2.3 (3H, *s*, C-3 OAc), 2.38 (3H, *s*, C-7 OAc), 3.88 (3H, *s*, C-6 OMe), 3.95 (3H, *s*, C-5 OMe), 7.0 (1H, *s*, C-8), 7.47 (3H, *m*, C-3′, 4′, 5′), 7.76 (2H, *m*, C-2′, 6′).

3, 5, 6, 7-*Tetramethoxyflavone* (**3**). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ 3.83 (3H, *s*, –OMe), 3.87 (3H, *s*, –OMe), 3.94 (3H, *s*, –OMe), 3.97 (3H, *s*, –OMe), 6.75 (1H, *s*, C-8), 7.45 (3H, *m*, C-3′, 4′, 5′), 8.02 (2H, *m*, C-2′, 6′).

5, 7-*Dihydroxy*-3, 6-*dimethoxyflavone* (**4**). The UV spectrum of this compound was identical with already published data [5].

3, 5, 7-*Trihydroxy*-8-*methoxyflavone* (**5**). Colour under UV: deep purple, no colour change with ammonia vapours. R_f values on Whatman paper MM3: 0.09 (15% HOAc), 0.83 (TBA). UV λ_{max}^{MeOH} nm: 274; 313 sh; 364; NaOMe: 286; 340 sh; 412; $AlCl_3$: 253; 280; 347; 423; $AlCl_3$–HCl: 253; 280; 347; 423; NaOAc: 283; 340 sh; 405; H_3BO_3: 274; 311 sh; 366. ^{1}H NMR (90 MHz, CCl_4, TMS): δ 3.85 (3H, *s*, C-8 OMe); 6.1 (1H, *s*, C-6), 7.4(3H, *m*, C-3′, 4′, 5′), 8.2 (2H, *m*, C-2′, 6′). EIMS (70 eV, probe) *m/z* (rel. int.): 300 (M^+, 100), 285($M^+ - CH_3$, 95), 282($M^+ - H_2O$, 17), 257($M^+ - CH_3CO$, 70), 139(A_1-28-15, 30), 105(B_2, 40).

3, 7, 8-*Trimethoxy*-5-*hydroxyflavone* (**6**). UV λ_{max}^{MeOH} nm: 274; 357; NaOMe: 277; 357; $AlCl_3$: 288; 335; 420; $AlCl_3$–HCl: 288; 335; 420; NaOAc: 276; 357; H_3BO_3: 276; 357. ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ 3.8–4.0 (9H, C-3, C-7, C-8 OMe), 6.4 (1H, *s*, C-6) 7.5 (3H, *m*, C-3′, 4′, 5′), 8.2 (2H, *m*, C-2′, 6′), offset: 12.3 (1H, *s*, C-5 OH).

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REFERENCES

1. Munz, P. A. (1968) *A California Flora*, p. 778. University of California Press.
2. Gottlieb, O. R. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.), p. 299. Chapman & Hall, London.
3. Wollenweber, E. and Dietz, V. H. (1979) *Phytochem. Bull.* **12**, 48.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, p. 264. Springer, Berlin.
5. Henricks, C. A. and Jefferies, P. A. (1964) *Aust. J. Chem.* **17**, 934.
6. Markham, K. R. and Mabry, T. J. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.), p. 74. Chapman & Hall, London.
7. Buschi, C. A., Pomillo, A. B. and Grox, E. G. (1980) *Phytochemistry* **19**, 903.
8. Asakawa, Y. (1971) *Bull. Chem. Soc. Jpn* **44**, 2761.
9. Wagner, H., Maurer, G., Farkas, L., Haensel, R. and Ohlendorf, D. (1971) *Chem. Ber.* **104**, 2381.
10. Krishnamurti, M., Seshadri, T. R. and Shankaran (1966) *Tetrahedron* **22**, 941.
11. Thomas, M. B. and Mabry, T. J. (1967) *J. Org. Chem.* **32**, 3254.