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J. Nat. Prod., **1994**, 57 (11), 1549-1551• DOI: 10.1021/np50113a012 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 11, pp. 1549-1551, November 1994

COUMARINS FROM *ASTEROLASIA TRYMALIOIDES*

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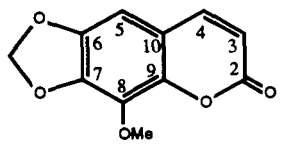
ABSTRACT.—A novel coumarin, 6,7-methylenedioxy-8-methoxycoumarin [**1**], and four known coumarins, umbelliferone, scopoletin, bergapten, and xanthotoxin, have been isolated from the aerial parts of *Asterolasia trymalioides*.

Asterolasia trymalioides (Rutaceae) F. Muell., a prostrate to erect shrub in alpine heaths, is a south-eastern Australian species (occurring in the Snowy Mountains of southern New South Wales and eastern Victoria) (1). Of 11 species of *Asterolasia* (2), three have recently been studied (3,4), as part of our ongoing chemotaxonomic survey of Australian Rutaceae. In this paper we wish to report on the major secondary metabolites found in a sample of the aerial parts of *A. trymalioides* and to discuss their chemotaxonomic significance.

Five coumarins have been isolated, four from an *n*-hexane extract and one from an EtOAc extract, by a combination of cc, circular tlc, and prep. tlc. They were characterized as umbelliferone (4–6), 6,7-methylenedioxy-8-methoxycoumarin [**1**], bergapten (4,7,8), xanthotoxin (4,7,8), and scopoletin (4–6). All except **1** were characterized by direct comparison of their physical and spectroscopic properties with published data (4–8) and co-tlc with commercially available samples.

The novel coumarin **1** was identified and characterized by spectroscopic methods. It was observed on tlc as a bluish-white fluorescent spot under uv light (366 nm) and showed uv absorption maxima at 321 and 231 nm, suggestive of a coumarin (9). The empirical formula was found to be $C_{11}H_8O_5$ from high-resolution eims data. The ^{1}H-nmr spectrum (Table 1) revealed the typical H-3 and H-4 protons of the coumarin nucleus (9). The chemical shift for H-4 (δ 7.56) indicated the absence of any oxygenation at C-5 (9). Three more signals indicated an aromatic proton, a methylenedioxy group, and a methoxyl substituent. As there was no oxygenation at C-5, the aromatic singlet must be assigned as H-5 and this was confirmed by an nOe difference experiment, where irradiation of the signal at δ 6.58 enhanced the signal for the H-4 proton. The ^{1}H-nmr chemical shift data for **1** differed significantly from those published for 7,8-methylenedioxy-6-methoxycoumarin (10), the most notable feature being the highly deshielded resonance for the



1

TABLE 1. ^{1}H-Nmr Data for the Coumarin **1**.

Position	δ ^{1}H[a]
3	6.29 (d, J=9.5 Hz)
4	7.56 (d, J=9.5 Hz)
5	6.58 (s)
8-OMe	4.12 (s)
-O-CH_2-O-	6.06 (s)

[a]Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 7.27 ppm; 400 MHz.

methoxyl, indicative of the steric hindrance encountered at C-8. The attachment of the methoxyl group at C-8 was further supported by an nOe experiment, since irradiating the methoxyl protons gave no enhancement of the H-5 signal. The methylenedioxy group must therefore be at the 6,7 positions and thus the structure of this coumarin can unambiguously be assigned as **1**, which is novel.

Inclusive of the present results, all four species of *Asterolasia* examined to date (3,4) have yielded coumarins. However, these show considerable structural diversity between species. *A. drummondii* yielded a range of 5,7-oxygenated-6,8-prenylated coumarins; *A. squamuligera* gave a 3-prenyl-7-oxygenated coumarin; *A. phebalioides* and *A. trymalioides* produce coumarins with 6-prenylation and oxygenation at the 5, 7, and 8 positions but with no 8-prenylation. Cyclization of the 6-prenyl group, leading to the formation of a furan but not a pyran ring system has been observed in *A. phebalioides* and *A. trymalioides* but not in the other two species. These furocoumarins and other oxygenated simple coumarins are also present in many species of the genus *Phebalium* (11). Thus, the co-occurrence of these coumarins in the genus *Phebalium*, and in extracts from two species of *Asterolasia*, lends some support to the taxonomic affinity between these two genera proposed by Armstrong's cladistic study (12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were recorded on Perkin-Elmer 781 and Perkin-Elmer 552 spectrophotometers, respectively. The ms was recorded on an AEI MS-902 spectrometer. ^{1}H nOe difference nmr spectra were recorded in $CDCl_3$ on a Bruker AMX-400 instrument. Chemical shifts were reported in ppm relative to $CHCl_3$. The following types of Si gel were used: Si gel (Merck 7734.1000) for cc and Si gel 60-PF_{254} for tlc. Sephadex LH-20 (Sigma 82 H 0368) was also used for cc. Authentic samples of bergapten, xanthotoxin, umbelliferone, and scopoletin were obtained from the Aldrich Chemical Company.

PLANT MATERIAL.—Aerial parts of *A. trymalioides* were collected from a specimen growing in the Australian National Botanic Garden (ANBG), in Canberra. A voucher specimen (CBG 8503595) representing this collection is housed in the ANBG Herbarium.

EXTRACTION AND ISOLATION.—Powdered aerial parts of *A. trymalioides* (32 g) were extracted in a Soxhlet with, successively, *n*-hexane, EtOAc, and MeOH. The concentrated *n*-hexane extract (0.9 g) was subjected to cc on Si gel, eluting with hexane containing increasing amounts of EtOAc. The fractions eluted with hexane-EtOAc (9:1) were mixed and subjected to further small cc for isocratic elution with hexane-EtOAc (3:1) to obtain bergapten and xanthotoxin as a mixture (1.8 mg). The later column fractions (15%–20% EtOAc in hexane) were combined and subjected to circular tlc, eluting with 25% EtOAc in hexane to give umbelliferone (1.4 mg) and **1** (1.7 mg) which were separated by prep. tlc (double development with 100% $CHCl_3$). The EtOAc extract (0.7 g) was subjected to cc over Sephadex LH-20 to remove chlorophylls. From the chlorophyll-free extract, scopoletin (2.3 mg) was isolated by prep. tlc ($CHCl_3$-EtOAc, 5:1).

6,7-Methylenedioxy-8-methoxycoumarin [**1**].—Amorphous; 1.7 mg; uv λ max (EtOH) 321 and 231 nm; ^{1}H nmr, see Table 1. Eims *m/z* $[M]^+$ 220.0384 (calcd 220.0372 for $C_{11}H_8O_5$); major fragment ions *m/z* 192 $[M-28]^+$ (62.3), 177 $[M-43]^+$ (19.8), 149 $[M-71]^+$ (16.4), 91 $[M-129]^+$ (10.5), and 79 $[M-141]^+$ (16.4).

Bergapten and xanthotoxin.—Amorphous; 1.8 mg. Eims *m/z* $[M]^+$ 216.1941 (calcd 216.193 for $C_{12}H_8O_4$); uv, ^{1}H nmr, eims in agreement with literature (4,7,8).

Scopoletin.—Prisms from EtOH; 2.3 mg; mp 204°. Eims *m/z* $[M]^+$ 192.1713 (calcd 192.171 for $C_{10}H_8O_4$); mp, uv, and ^{1}H nmr in agreement with literature (4–6).

Umbelliferone.—Needles from H_2O, 1.4 mg; mp 230°–232°. Eims *m/z* $[M]^+$ 162.0312 (calcd 162.0317 for $C_9H_6O_3$), uv, ir, ^{1}H nmr, eims in agreement with literature (4–6).

ACKNOWLEDGMENTS

One of us (S.D.S.) thanks the Association of Commonwealth Universities for the award of a scholarship. Nmr studies were performed in the University of Strathclyde nmr laboratory.

LITERATURE CITED

1. J.A. Armstrong, in: "Rutaceae. Plants of New South Wales: A Census of the Cycads, Conifers and Angiosperms." Ed. by S.W Jacobs and J. Pickard, Royal Botanic Gardens, Sydney, 1981, p. 191.

2. J.A. Armstrong and I.R. Telford, "Asterolasia, Flora of South Australia," Government Printers, Adelaide, **4**, 768 (1986).
3. S.D. Sarker, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *J. Nat. Prod.*, **57**, 324 (1994).
4. S.D. Sarker, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Biochem. Syst. Ecol.*, **22**, 433 (1994).
5. D. Brown, R.O. Asplund, and V.A. McMahon, *Phytochemistry*, **14**, 1083 (1975).
6. R.H. Abu-Eittah and B.A.H. El-Tawil, *Can. J. Chem.*, **63**, 1175 (1985).
7. K.H. Lee and T.O. Soine, *J. Pharm. Sci.*, **58**, 681 (1969).
8. J.P. Kutney, A.K. Verma, and R.N. Young, *Tetrahedron*, **28**, 5091 (1972).
9. R.D.H. Murray, J. Mendez, and S.A. Brown, "The Natural Coumarins: Occurrence, Chemistry, and Biochemistry," John Wiley and Sons Ltd., Chichester, UK, 1982.
10. R.D.H. Murray and M. Stefanovic, *J. Nat. Prod.*, **49**, 550 (1986).
11. M.A. Quader, J.A. El-Turbi, J.A. Armstrong, A.I. Gray, and P.G. Waterman, *Phytochemistry*, **31**, 3083 (1992).
12. H.M. Stace, J.A. Armstrong, and S.H. James, *Plant Syst. Evol.*, **187**, 1 (1993).

Received 18 March 1994