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COUMARINS FROM *TRIPHASIA TRIFOLIATA*

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Key Word Index—*Triphasia trifoliata*; Rutaceae; coumarins; isomeranzin; umbelliferone; triphasiol.

Abstract—A methanolic extract of the leaves of *Triphasia trifoliata* contained isomeranzin, umbelliferone and 7-(3′-methyl-2′,3′-dihydroxybutyloxy)-8-(3″-methyl-2″-oxobutyl) coumarin (triphasiol).

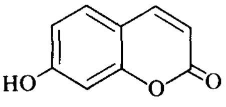
INTRODUCTION

Many rutaceous plants have been used in ethnic medicine [1] and it is possible that coumarins are responsible for many of their properties. In the course of a continuing search for biologically active compounds from plants, particularly those belonging to the family Rutaceae, several members of this family have been investigated [2–6]. In the present communication, we report the characterization of three coumarins isolated from *Triphasia trifoliata.*

RESULTS AND DISCUSSION

The leaves of the plant were extracted with hot MeOH and the Et_2O-soluble portion of this extract was chromatographed on a silica gel column. The first compound eluted was isomeranzin (**2**) which was identified by the comparison of its physical and spectral data (UV, IR, ^{13}H NMR) with those of an authentic sample. This coumarin has been isolated previously from *Skimmia japonica* Thumb [7].

From the next fraction, umbelliferone (**1**) was isolated and identified by comparison with an authentic sample.



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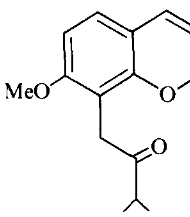

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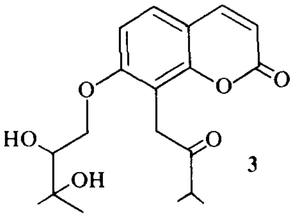


3

The subsequent fractions were combined and purified further by preparative TLC, to yield the third coumarin, triphasiol (**3**) (mp 85°) which showed a green fluorescence in UV light. Its IR spectrum showed a band at 1720 cm^{-1}, typical of a coumarin lactone and another band at 1700 cm^{-1} attributable to a carbonyl absorption. It also showed the presence of OH and aromatic absorptions at 3300 and 1600 cm^{-1}, respectively. Additional evidence for the presence of a coumarin lactone comes from the ^{1}H NMR spectrum which showed a typical AB system (δ 6.15, H-3; δ 7.65, H-4; $J_{3,4} = 10$ Hz) for the protons C3, C4. The chemical shift (δ 7.65) assigned to the C-4 proton indicated the absence of an alkyl or alkoxyl substituent at C-5 as a substituent at C-5 would result in a downfield shift of the C-4 proton to *ca* δ 8 [8, 9]. Thus, the signal at δ 7.35 was assigned to a proton at C-5. The spectrum also showed a pair of doublets, one at δ 7.35, $J = 10$ Hz, assignable to the C-5 proton and the other at δ 6.85, $J = 10$ Hz, which are typical of *ortho* protons in a 1, 2, 3, 4-substituted benzene ring [9, 10]. Therefore the signal at δ 6.85 was assigned to the proton at C-6. The C-5 and C-6 proton assignments were further supported by the absence of a singlet which could be assigned to a C-8 proton and the lack of C-6-mono-oxygenated coumarins in nature [11]. The upfield shift (δ 6.85) of the C-6 proton is characteristic of an aromatic proton adjacent to an oxygen substituent [12, 13].

In view of this evidence and since nearly all naturally occurring coumarins possess an oxygen substituent at C-7 [14], a part of the side-chain moiety could be linked to C-7 through an oxygen atom. Additional ^{1}H NMR signals were observed at δ 4.05 (2 H, $-CH_2$ group adjacent to an aromatic system and a carbonyl group), δ 2.8 (1 H, –CH proton attached to a tertiary carbon adjacent to a keto group), δ 4.2 (2 H, adjacent to –O–Ar) and δ 2.8 (1 H, attached to C–OH).

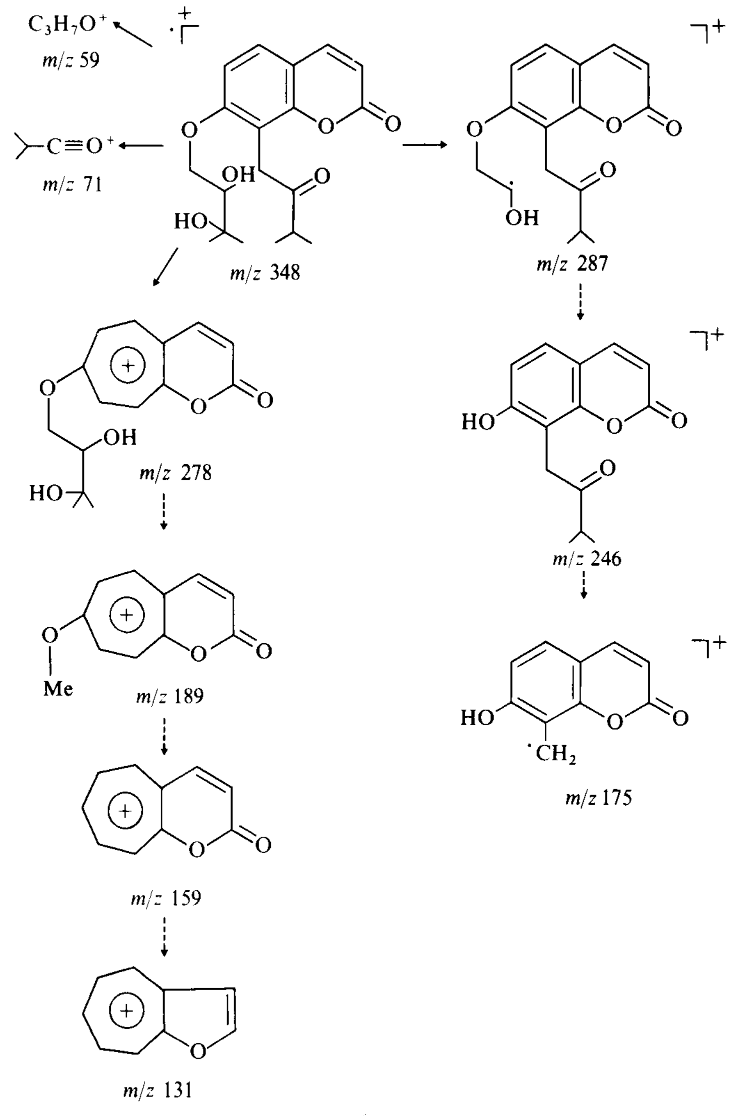
The mass spectrum of **3** showed a M^+ at m/z 348 and other peaks corresponding to the loss of fragments from the side-chain moiety such as m/z 333 ($M^+ - Me$), 331 ($M^+ - OH$) and 304 [$M^+ - Me_2, CH, H$] were observed. Peaks due to the fragmentation of the coumarin moiety, namely, m/z 159, 146, 131, 118, 91 were also observed. Scheme 1 describes the major ions present in the mass spectrum.

The structure of **3** was confirmed by comparing the spectral data with those of the diol obtained by the treatment of isoponcimerin with oxalic acid in boiling water. Although this coumarin has been described in the literature [15], as far as we are aware, this is the first instance where this compound has been obtained from natural sources. Triphasiol is not an artefact, formed during the processing of the extract, as we have shown that it is present in the original methanolic extract.

EXPERIMENTAL

T. trifoliata DC. was collected in Sept. 1979 at Nawinna near Colombo, Sri Lanka. Identity of compounds was established by mp, mmp, TLC and IR. ^{1}H NMR spectra were recorded at 100 MHz unless otherwise specified and are given in δ (Hz) relative to TMS; mps, determined in open capillaries, are uncorr.

Isolation. Dried, powdered leaves of *T. trifoliata* (800 g) were extrd with hot MeOH for 3 days. Evapn of solvent gave a brown solid (5 g) which was dissolved in Et_2O, washed with H_2O, dried (Na_2SO_4) and the Et_2O evapd. A brown solid (3 g) was obtained, which was chromatographed on Si gel. Fractions from the column were monitored by TLC (Si gel, $CHCl_3$–MeOH, 50:1). The fraction which was eluted with C_6H_6–$CHCl_3$ (1:1) contained a blue fluorescent compound which on crystallization from hexane yielded isomeranzin (**2**) (20 mg), mp 60°. The next fractions eluted with C_6H_6–$CHCl_3$ (1:3) gave umbelliferone (30 mg) mp 232°. Elution with $CHCl_3$ gave **3**, which was further purified by prep. TLC (Si gel, $CHCl_3$–MeOH, 40:1) and on crystallization from $CHCl_3$–petrol yielded **3** (15 mg) mp 85°. (Found: M^+, 348.152. $C_{19}H_{24}O_6$ requires: M^+, 348.157); MS *m/z* (rel. int.) 348 (7), 334 (2), 304 (3), 289 (19), 278 (11), 259, 260, 246 (39), 232, 219 (32), 202 (55), 189 (46), 176 (77), 175 (55), 160, 159 (3), 146 (14), 131 (12), 118 (10), 91 (17), 90 (11), 71, 59 (100), 43 (75). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1715, 1602, 1590, 1480. ^{1}H NMR (100 MHz, $CDCl_3$): δ 7.65 (1 H, *d*, J = 10 Hz) H-4; 7.35 (1 H, *d*, J = 10 Hz) H-5; 6.85 (1 H, *d*, J = 10 Hz) H-6; 6.2 (1 H, *d*, J = 10 Hz) H-3; 4.2–3.8 (5 H, *m*) 2H-1″. H-2′; δ 3.7–2.8 (2 H)-OH; δ 2.8 (1 H, *septet*) H-3″; δ 1.3–1.12 (12 H, *m*) 4-Me.



Scheme 1.

REFERENCES

1. Usher, G. (1971) *A Dictionary of Plants Used by Man*. Constable, London, and refs. cited therein.
2. Gray, A. I. and Waterman, P. G. (1978) *Phytochemistry* **17**, 845.
3. De Silva, L. B., De Silva, U. L. L., Mahendran, M. and Jennings, R. C. (1979) *Phytochemistry* **18**, 1255.
4. De Silva, L. B., De Silva, U. L. L., Mahendran, M. and Jennings, R. C. (1980) *Phytochemistry* **19**, 2794.
5. De Silva, L. B., De Silva, U. L. L., Mahendran, M. and Jennings, R. C. *J. Nat. Council Sri Lanka* (in press).
6. De Silva, L. B., De Silva, U. L. L., Mahendran, M. and Jennings, R. C. *Indian J. Chem.* (in press).
7. Atkinson, E., Boyd, D. R. and Grundon, M. F. (1974) *Phytochemistry* **13**, 853.
8. Fisher, J. F. and Nordby, H. E. (1965) *J. Food Sci.* **30**, 869.
9. Nielsen, B. E. and Lemmich, J. (1964) *Acta Chem. Scand.* **18**, 1379.
10. Jackman, L. M. (1959) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*. Pergamon Press, Oxford.
11. Austin, D. J. and Meyers, M. B. (1965) *Phytochemistry* **4**, 245.
12. Bredenberg, J. B. and Shoolery, J. N. (1961) *Tetrahedron Letters* 285.
13. Bottomley, W. (1963) *Aust. J. Chem.* **16**, 143.
14. Dean, F. M. (1963) *Naturally Occurring Oxygen Ring Compounds*. Butterworth, London.
15. Guiotto, A., Rodighiero, P., Pastorini, G. and Celon, E. (1977) *Phytochemistry* **16**, 1257.

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2,5-DIMETHYLCOUMARINS FROM LEAVES OF *JUNIPERUS SABINA*[*]

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Key Word Index—*Juniperus sabina*; Cupressaceae; new coumarins; coumarsabin; 8-methoxycoumarsabin; siderin.

The essential oil and extracts of *Juniperus sabina* L. were used in folk medicine as an abortive and a purgative, but its potency and hazardous effects prohibited its use. Some studies on the composition of this plant have been carried out [1–3], but no report on the existence of coumarins appears to have been made. In this paper we describe the identification of siderin (**1**) and the structure determination of coumarsabin (**2**) and 8-methoxycoumarsabin (**4**), two new coumarins with the previously undescribed 2,5-dimethyl substitution pattern.

Isolations were performed by chromatography and crystallization from a carefully defatted fraction of the hexane extract, which also contained some lignans and terpenoids. Siderin (**1**) showed MS, IR and UV spectra closely similar to those described [4, 5] and a ^{1}H NMR spectrum superimposable with that of an authentic sample.

Coumarsabin (**2**), mp 86–87°, showed MS with M^+ at m/z 234 ($C_{13}H_{14}O_4$). In the IR spectrum it presented the typical absorptions of methoxycoumarins at 1710, 1610, 1165, 1060 and 840 cm^{-1} and in the UV $\lambda_{\max}^{\mathrm{EtOH}} = 230$, 241 *sh*, 299 *sh* and 321 nm. The ^{1}H NMR spectrum contained singlets of two Me groups at δ 2.14 and 2.65, two aromatic OMe groups at δ 3.82 and 3.87 and a broad singlet of two aromatic protons at δ 6.66. The comparison with the spectrum of siderin has led us to propose the structure **2** for coumarsabin. The benzene and Eu(fod)$_3$-induced shifts in the ^{1}H NMR spectrum, which mostly agree with previous results in these fields [6, 7], are shown in Table 1. Degradation of **2** to the propiophenone derivative **3** [8] confirms the presence of the 3-Me group. The *m*-coupling constant of 3 Hz between the aromatic protons, the small *o*- and *p*-benzylic couplings observed in the ^{1}H NMR spectrum of **3**, its benzene-induced shifts (Table 1) and the IR and UV absorptions confirm structure **2** for coumarsabin.

Table 1. Benzene and Eu(fod)$_3$-induced shifts

2	3-Me	4-OMe	5-Me	6-H	7-OMe	8-H
$\Delta_{C_6D_6}^{CDCl_3}$ (ppm)	0.14	0.66	0.26	0.15	0.60	0.15
$\Delta_{Eu(fod)_3}^{*}$	1.00	0.31	0.23	0.17	0.03	0.17
3	α-CH$_2$	β-Me	3′-H	4′OMe	5′-H	6′-Me
$\Delta_{C_6D_6}^{CDCl_3}$ (ppm)	0.62	0.22	0.08	0.51	0.10	0.54

* Relative to 3-Me (shifted 2.31 ppm down-field).

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[*] For the preceding paper in this series see J. de Pascual *et al.* (1978) *An. Quim.* **74**, 1093.