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COUMARINS AND CHROMONES FROM *LOMATIUM MACROCARPUM**†

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Key Word Index—*Lomatium macrocarpum*; Umbelliferae; coumarins; osthol; macrocarpin; sibiricin; chromones; peucenin methyl ether.

Abstract—Roots of *Lomatium macrocarpum* (Hook. & Arn.) C. & R. yielded osthol (7-methoxy-8-[3-methyl-2-butenyl]-coumarin) and a chromone, 2-methyl-5-hydroxy-6-[3-methyl-2-butenyl]-7-methoxychromone, identified spectroscopically and by synthesis. The aerial parts of the plant also contained this chromone along with sibiricin (5,7-dimethoxy-8-[3-methyl-2,3-epoxybutyl]-coumarin) and a new coumarin named macrocarpin. By spectroscopy and chemical degradation macrocarpin was shown to be 7-methoxy-8-(3-methyl-4-[2-methyl-*cis*-2-butenoyloxy]-*cis*-2-butenyl)coumarin. These products were not found in four other *Lomatium* species examined.

INTRODUCTION

THE GENUS *Lomatium* (family Umbelliferae), the subject of much taxonomic revision, is represented on the arid plains of North America by several dozen species, and many additional species are found on the Pacific coast. *L. columbianum* Mathias & Const.,[1] *L. dissectum* var. *multifidum* (Nutt.) Mathias & Const.,[2] *L. nuttallii* (A. Gray) Macbr.[3] and *L. suksdorfii* (Wats.) C. & R.[4] have been examined chemically and have been found to contain coumarin derivatives of various kinds. *L. macrocarpum* (Hook. & Arn.) C. & R. (*Cogswellia macrocarpa* (Nutt.) M. E. Jones) is a short-stemmed species with a thick, deep tap root, and is found throughout the plains and further west. It has not been examined previously.

RESULTS AND DISCUSSION

From the roots of flowering plants were isolated osthol and a larger amount of a non-fluorescing compound, m.p. 106–108°, whose [^{1}H]NMR spectrum showed apparent doublets ($J \sim 0{\cdot}5$ Hz) at $\delta 2{\cdot}33$(3H) and 6·00(1H) typical of 2-methylchromones. A hydroxyl proton at $\delta 12{\cdot}74$ indicated the presence of a chelated HO group at C-5; a methoxyl group ($\delta 3{\cdot}87$) was assigned to C-7 on biogenetic and UV spectroscopic grounds. A 3-methyl-2-butenyl substituent at C-6 or C-8 was indicated by signals at $\delta 1{\cdot}67$ and 1·78 (3H each, *d*, *J* 1 Hz), 5·20 (1H, *qt*, *J* 1, 8 Hz resp.) and 3·33 (2H, *d*, *J* 8 Hz). An aromatic singlet at $\delta 6{\cdot}34$ was the remaining signal. Thus the compound was either heteropeucenin methyl ether (m.p. 110°)[5]

* Part II in the series "Prairie Plants". For Part I see STECK, W. (1970) *Phytochemistry* **9**, 1145.
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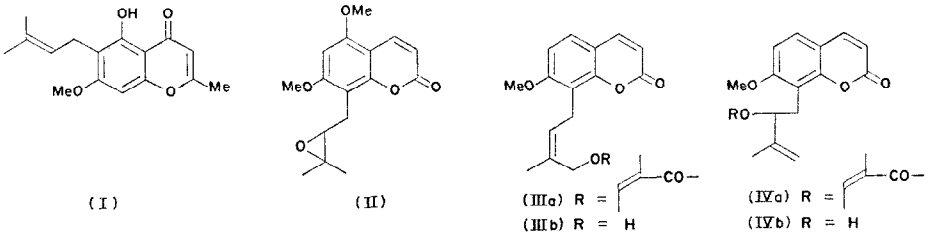
[1] WILLETTE, R. E. and SOINE, T. O. (1964) *J. Pharm. Sci.* **53**, 275.
[2] GUPTA, K. P. and SOINE, T. O. (1964) *J. Pharm. Sci.* **53**, 1543.
[3] LEE, K.-H. and SOINE, T. O. (1968) *J. Pharm. Sci.* **57**, 865.
[4] WILLETTE, R. E. and SOINE, T. O. (1962) *J. Pharm. Sci.* **51**, 149.
[5] DEAN, F. M. and TAYLOR, D. A. H. (1966) *J. Chem. Soc.* **C**, 114.

or peucenin methyl ether (I) (m.p. 109°).[6] Its identity with the latter was shown by chromatographic, spectral and m.m.p. comparisons with a synthetic sample prepared by the action of diazomethane on peucenin.[7] This is the second reported natural occurrence of this chromone derivative, Dean and Robinson[6] having recently isolated it from the heartwood of the rutifer *Cedrelopsis grevei*.



(I) (II) (IIIa) R = (IIIb) R = H (IVa) R = (IVb) R = H

The aerial parts of the plant contained only traces of osthol but significant amounts of (I). In addition two major coumarins were isolated. One, m.p. 151–153°, $[\alpha]_D^{25}$ +58·7° (1·8, $HCCl_3$), fluoresced brilliant blue under UV light and had λ_{max}^{EtOH} at 328 nm, suggesting a 5,7-dioxygenated coumarin. The NMR spectrum agreed in all respects with the published spectrum of sibiricin (II) (m.p. 152–153°).[8] The coumarin is therefore sibiricin, since aculeatin, the only isomer with a structure consistent with these UV and NMR spectra, has m.p. 113°.[9]

The other coumarin, $C_{20}H_{22}O_5$, m.p. 71–73·5° $[\alpha]_D^{25}$ ±0·0°, was a novel compound for which the trivial name *macrocarpin* is proposed. It fluoresced violet under UV light and exhibited an absorption maximum at 320 nm. Its structure was deduced from the [^{1}H]NMR spectrum. Doublets (J 9·5 Hz) at δ6·20 and 7·59 arose from H-3 and H-4 of the coumarin nucleus, and H-5 and H-6 were also present (doublets at δ7·28 and 6·80, J 9·0 Hz). A methoxyl group (δ3·90) was assigned to C-7, in keeping with the UV absorption and fluorescence, and all other signals to the substituent at C-8. These included a doublet at δ3·61 (2H) and a triplet at 5·50 (1H), both J 8 Hz, the latter with a small additional coupling, $J \sim 1$ Hz; a singlet at δ4·92 (2H); a doublet at 1·73 (3H) ($J \sim 1$ Hz); and the typical signals of an angelyl ester at δ1·88, 1·97 and 6·04. For these data, structure (IIIa) is the only acceptable formula. The alternative structure (IVa) would be expected to show multiple signals for the terminal methylene protons,[10,11] and was definitely excluded by examination of the hydrolysis product of macrocarpin.

Attempted acid hydrolysis of macrocarpin using aqueous methanolic 20% H_2SO_4 yielded only unchanged starting material. Alkaline hydrolysis with 7% aqueous methanolic NaOH, on the other hand, proceeded very readily, affording 2-methyl-2-butenoic acid and a coumarin (IIIb) whose NMR spectrum lacked angelyl protons. This spectrum ($DCCl_3$) showed the same aromatic, methoxyl and benzylic CH_2 signals as for macrocarpin, along with a sharp 2H singlet at δ4·41, a 3H doublet at 1·80 ($J \sim 1$ Hz), a broad hydroxyl singlet at 1·9 and a triplet (J 8 Hz) with a slight additional splitting ($J \sim 1$ Hz) at δ5·22. The chemical shift of this triplet is too great to accommodate structure (IVb), but is entirely suitable for

[6] DEAN, F. M. and ROBINSON, M. L. (1971) *Phytochemistry* **10,** 3221.
[7] JAIN, A. C., LAL, P. and SESHADRI, T. R. (1969) *Indian J. Chem.* **7,** 1072.
[8] AUSTIN, P. W., SESHADRI, T. R., SOOD, M. S. and VISHWAPAUL (1968) *Tetrahedron* **24,** 3247.
[9] DUTTA, P. (1942) *J. Indian Chem. Soc.* **19,** 425.
[10] STANLEY, W. L., WAISS, A. C., LUNDIN, R. E. and VANNIER, S. H. (1965) *Tetrahedron* **21,** 80.
[11] BASA, S. C., CHATTERJEE, J. and CHATTERJEE, A. (1971) *Tetrahedron Letters* 1977.

the olefinic proton in (IIIb). Moreover, (IVb) is auraptenol,[10] a known coumarin whose published NMR spectrum differs markedly from that of (IIIb). The protons of macrocarpin's side chain give NMR signals similar to those obtained from 7-acetoxy-8-(3-methyl-4-acetoxy-2-butenyl)coumarin, a compound synthesized[12] from 7-acetoxy-8-(3-methyl-2-butenyl)coumarin. The somewhat lower field of the esterified methylene in macrocarpin (δ4·9 vs 4·4 in the synthetic model) is expected because of greater deshielding from angelate than from acetate.

Unequivocal confirmation of structure (IIIb) was obtained when the NMR spectrum of this hydrolysis product of macrocarpin was measured in deuterated dimethylsulphoxide, in which solvent the hydroxyl group appeared as a triplet (1H) (*J* 5·5 Hz) at δ4·5 and the coupled methylene group as a doublet at 4·19. The multiplicity of other signals remained unchanged from the spectrum in deuterochloroform. Macrocarpin is therefore 7-methoxy-8-(3-methyl-4-[2-methyl-*cis*-2-butenoyloxy]-2-butenyl)coumarin. Application of the Nuclear Overhauser Effect[13,14] indicated the butenyl sidechain of macrocarpin has the *cis*-configuration. Saturation of the methylene resonance at δ3·61 increased the intensity of the methylene peak at δ4·92 but left the methyl protons at δ1·80 unaffected. Conversely, saturation of the olefinic proton at δ5·50 increased the methyl intensity but not the intensity of the methylene at δ4·92. This indicates the two methylenes are *cis* to each other, and that the olefinic proton is *cis* to the 3-methyl group.

The formula (IIIa) for macrocarpin was supported by the MS which showed a molecular ion peak at *m/e* 342 (3%), a base peak at 242 resulting from loss of angelic acid, a peak at 259 (24%) from loss of angelyl ion, and further peaks characteristic of 7-methoxycoumarins[15] at *m/e* 189 (58%), 159 (7%) and 131 (29%). The base peak showed fragmentation to ions of *m/e* 227 (14%) and 211 (63%). Strong peaks at *m/e* 83 (90%) and 55 (81%) indicated the presence of angelyl and 2-butenyl ions respectively. Appropriate metastable peaks were found for most of these transformations.

Several other *Lomatium* species were also examined, but macrocarpin could not be detected in any of these. *L. columbianum* Mathias & Const. roots gave columbianetin angelate, as reported,[1] but the shoots contained only trace quantities of coumarins. *L. utriculatum* (Nutt.) C. & R. aerial parts yielded no significant quantities of coumarins, nor did shoots or roots of *L. villosum* Raf. (*L. foeniculaceum* C. & R.). *L. nudicaule* (Pursh) C. & R. roots gave trace quantities of angelicin and osthol, along with considerable β-sitosterol. From these limited studies it would appear that coumarins and chromones are not of uniform occurrence throughout this genus, but that accumulation of substantial amounts of these compounds can occur in some species. Macrocarpin in particular, with an oxygen function at the terminal position of the sidechain, represents a new type of coumarin of some biogenetic interest. The study of its formation may yield general insights into the biosynthesis of this type of sidechain in natural products.

EXPERIMENTAL

Plant materials. Lomatium columbianum was collected east of Portland, Oregon, in April 1970, and identified by Prof. B. G. Brehm, Botany Department, Reed College, Portland, Oregon. *L. macrocarpum* was collected in May 1971 and in May and June 1972 just north of Saskatoon, Saskatchewan; *L. nudicaule* in June

[12] BOHLMANN, F. and FRANKE, H. (1971) *Chem. Ber.* **104,** 3229.
[13] TOMIMATSU, T., HASHIMOTO, H., SHINGU, T. and TORI, K. (1969) *J. Chem. Soc.* **D,** 168.
[14] OHTSURU, M., TERAOKA, M., TORI, K. and TAKEDA, K. (1967) *J. Chem. Soc.* **B,** 1033.
[15] NIELSEN, B. E. (1970) *Coumarins of Umbelliferous Plants,* Chap. 3, Royal Danish School of Pharmacy, Copenhagen.

1968 near Ladner, British Columbia; *L. villosum* in May 1970 and May 1972 in Saskatoon. These species were identified by Dr. Vernon Harms, University of Saskatchewan, Herbarium, Saskatoon. *L. utriculatum* was collected in June 1968 on Vancouver Island, by Prof. B. A. Bohm, Botany Department, University of British Columbia. These species were identified by Prof. W. M. C. Taylor at the same Department.

Extraction. The following examination procedure for *L. macrocarpum* was essentially that used for all these plants. Roots (80 g dry wt) were boiled and homogenized in EtOH and filtered; the filtrate was evaporated and the residue taken up in 80% aq. MeOH, which was then extracted 1 × with hexane, concentrated to remove MeOH and finally extracted with $CHCl_3$. This $CHCl_3$ extract (3·0 g) was chromatographed on 100 g (3 × 35 cm column) silicic acid, developing with 1:1, ether–hexane, collecting 20 ml fractions.

Osthol. Fractions 11–14 showed, on GLC, a peak with the retention time of osthol. Crystallization from alcohol gave needles, m.p. 83–84° (lit.[16] m.p. 84°). The compound's UV and NMR spectra[17] confirmed its identity.

2-Methyl-5-hydroxy-6-[3-methyl-2-butenyl]-7-methoxychromone (I). Fractions 9–12 contained a crystalline material, m.p. 106–108° from aq. alcohol. λ_{max} (EtOH) 258, 292 nm. The NMR spectrum (see above) was measured in $DCCl_3$ (TMS standard) using a Varian HA-100 spectrometer, and suggested structure (I). An authentic sample, prepared from peucenin (2-methyl-5,7-dihydroxy-6-[3-methyl-2-butenyl]chromone)[7] and diazomethane in $CHCl_3$–MeOH, proved identical in UV and NMR spectra and also in chromatographic properties to the natural material. A m.m.p. showed no depression (106–108°). Column chromatography of an extract from 200 g dry flowering aerial parts gave a small amount (25 mg) of (I).

Sibiricin (II). Fractions 74–79 deposited 0·15 g colourless needles, m.p. 151–153° from MeOH, $[\alpha]_D^{25}$ +58·7° (1·78, $HCCl_3$), λ_{max} (EtOH) 328 nm, unchanged by addition of alkali. Alcoholic solutions of the compound fluoresced brilliant blue. The NMR spectrum ($DCCl_3$) showed coumarin H-3/H-4 doublets (*J* 9·5 Hz) at δ6·10 and 7·95, the position of the latter indicating an oxygen function at C-5. Two methoxyl signals, at δ3·92 (6H), must arise from 5,7 substituents, the only positions admissible on UV and fluorescence grounds. An aromatic singlet at δ6·35 was present, and the remaining signals (3H singlets at 1·26 and 1·47 and an ABC multiplet (3H) near 2·97) required a 2,3-epoxy-3-methylbutyl residue.[18] The compound's m.p. indicated that the aromatic proton was located at C-6 (sibiricin, lit.[8] m.p. 152–153°) and not at C-8 (aculeatin, lit.[9] m.p. 113°).

Macrocarpin (IIIa). Fractions 15–19 deposited 0·11 g needles, m.p. 71·0–73·5° from ether hexane. (Found: C, 69·9; H, 6·6%. $C_{20}H_{22}O_5$ requires: C, 70·1; H, 6·5%) $[\alpha]_D^{25}$ ±0·0°. λ_{max} (EtOH) 247, 257, 320 nm. Alcoholic solutions had a violet fluorescence. The NMR spectrum was very similar to that of osthol except for the lack of one terminal methyl and the presence of additional methylene and angelate signals; for interpretation, see above. The ester group was recognized as angelate by its NMR signals: at δ6·04 a quartet of quartets (*J* 7, 1 Hz) due to the olefinic proton, at 1·88 a quartet (*J* 1 Hz) arising from the 2-methyl group, and at 1·97 a double quartet (*J* 7, 1 Hz) indicating the end methyl. High resolution MS provided these fragment formulas: *m/e* 342·1461 ($C_{20}H_{22}O_5$ requires: 342·1467), *m/e* 259·0970 ($C_{15}H_{15}O_4$ requires: 259·0970), *m/e* 242·0945 ($C_{15}H_{14}O_3$ requires: 242·0943).

Hydrolysis of macrocarpin. 20 mg macrocarpin were kept 18 hr in cold 7% aq. methanolic NaOH. The alcohol was evaporated in a stream of air and the remaining solution was carefully acidified with dil. HCl and extracted (CH_2Cl_2). This solution was shaken with 5% $NaHCO_3$, washed with H_2O, dried and evaporated to give 11 mg (IIIb), m.p. 105·5–107·0°, purified by gas chromatography (5% SE30 column, 200°) and identified by NMR as described above. The $NaHCO_3$ fraction was acidified and extracted with CH_2Cl_2, from which solution was identified directly a mixture of angelic and tiglic acids (GLC, with authentic standard, FFAP column) and after diazomethane treatment a mixture of methyl angelate and tiglate (UC-W98 column, 100° and 5% SE30 column, 140°). Isomerization of angelic to tiglic acid under hydrolysis conditions is well known (for example see Ref. 1).

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[16] SPÄTH, E. and PESTA, O. (1934) *Chem. Ber.* **67,** 853.
[17] STECK, W. and BAILEY, B. K. (1969) *Can. J. Chem.* **47,** 3577.
[18] STECK, W. and MAZUREK, M. (1972) *Lloydia* **35,** 418.