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THE STRUCTURE OF TWO NEW COUMARINS FROM THE ROOTS OF *LOMATIUM COLUMBIANUM**

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Key Word Index—*Lomatium columbianum*; Umbelliferae; coumarins; *trans-p*-coumaroyllomatin; *cis-p*-coumaroyllomatin; columbianadin; selinidin; lomatin; dihydrooroselol.

Abstract—Two new coumarins, obtained from the roots of *Lomatium columbianum* are shown to be *trans-p*-coumaroyllomatin and *cis-p*-coumaroyllomatin. Furthermore, the roots afforded columbianadin, selinidin, lomatin, (+)-dihydrooroselol, and a mixture of monoesters of khellactone.

INTRODUCTION

Lomatium columbianum Mathias et Const. has previously been an object of chemical investigations. In 1956 Call and Green [1] investigated this plant and from a petroleum ether extract they isolated a crystalline substance named colubianin. No structural studies were reported on this substance. In 1964 Willette and Soine [2] isolated columbianadin (**3**) and a glycoside, columbianin (**8**) [3,4] from petroleum ether and alcoholic extracts, respectively.

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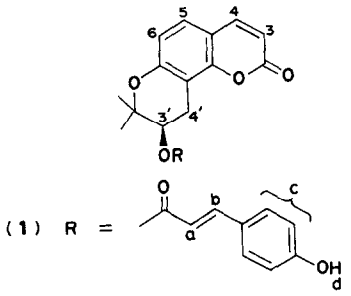
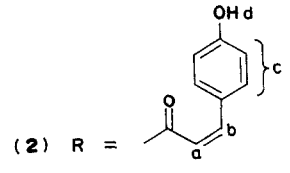
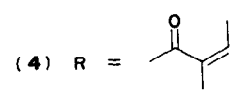
RESULTS AND DISCUSSION

In our hands the ether extract of the roots, in addition to columbianadin (**3**), afforded two new coumarins (**1**) and (**2**), selinidin (**4**), lomatin (**5**), (+)-dihydrooroselol (**6**), and a mixture of polar coumarins believed to be monoesters of khellactone (**7**), the acid moieties being angelic, senecioic, and isovaleric acid.

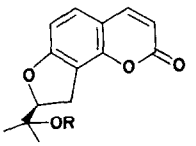
All attempts to crystallize the coumarins (**1**) and (**2**) were unsuccessful. (**1**) appears as a colourless, glassy substance whereas (**2**) is a colourless, amorphous powder. From the UV, IR, and especially ^{1}H NMR spectra (**1**) and (**2**) appeared to be esters of lomatin (**5**) with *trans-p*-coumaric acid and *cis-p*-coumaric acid, respectively. The proton signals arising from the acid moieties of (**1**) and (**2**) were shown to be concordant with those from the





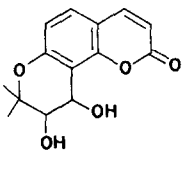


(**5**) R = H



(**6**) R = H

(**8**) R = β-D-gentiobiosyl



(**7**)

acid moieties of the methyl esters of authentic *trans-p*-coumaric acid and *cis-p*-coumaric acid, respectively. The *cis-p*-coumaric acid was obtained by photochemical inversion of the *trans* isomer.

Furthermore, saponification of (**1**) and (**2**) afforded lomatin (**5**) and in addition (**1**) yielded *trans-p*-coumaric acid whereas (**2**) gave a mixture of *cis*- and *trans-p*-coumaric acid.

The absolute configuration of lomatin (**5**) has been shown to be (*R*) [5] and accordingly the coumarins (**1**) and (**2**) are 3′(*R*)3′-0-((*E*)-(3-(4-hydroxyphenyl)propenoyl)-3′,4′-dihydroseselin and 3′(*R*)-3′-0-((*Z*)-(3-(4-hydroxyphenyl)propenoyl)-3′,4′-dihydroseselin, respectively.

The possibility of (**1**) or (**2**) being an artifact formed by inversion of the other isomer during the isolation procedure has been considered. TLC of a newly prepared CH_2Cl_2 extract of the roots disclosed that the extract contained both compounds. Accordingly (**1**) and (**2**) are believed to be naturally occurring.

The coumarins columbianadin (**3**), selinidin (**4**), lomatin (**5**), and (+)-dihydrooroselol (**6**) were identified by comparison with authentic materials.

EXPERIMENTAL

Mp's, IR and ^{1}H NMR spectra were determined as in a previous paper [6]. UV spectra were recorded in ethanol. TLC was carried out using Si gel GF_{254}, Merck, as the adsorbent. Si gel (Merck, 0.05–0.20 mm) used for column chromatography was treated as earlier described [6].

Plant material. Dried and ground roots of *Lomatium columbianum* Mathias et Const. were delivered by professor T. O. Soine (see lit. [2]).

Isolation and gross fractionation of the coumarin mixture. The dried and ground roots (800 g) on extraction with Et_2O and subsequent evaporation of the solvent afforded 24 g of an oily residue, which was dissolved in 90% MeOH and freed of lipids by extraction with petrol. The defatted extract (10 g) was chromatographed on Si gel (250 g). The eluent was CH_2Cl_2–CCl_4 (2:1) to which EtOAc was gradually added in amounts increasing to 20%, after which MeOH (0.5 5%) was added. The gross fractionation divided the defatted extract into four main fractions, A, B, C, and D mentioned in the order eluted.

Fraction A (2.1 g) was chromatographed on Si gel using C_6H_6 to which increasing amounts of EtOAc were added. The following compounds were obtained: a. Columbianadin (**3**) (1.1 g). Recrystallized from Et_2O mp 118.5–119°, $[\alpha]_D^{24}$ + 225° ($CHCl_3$; *c* 1.0) (lit. [7] mp 118.5–119°, $[\alpha]_D^{26}$ + 227°). The ^{1}H NMR and IR spectra were concordant with those of an authentic sample. b. A mixture (120 mg) of two blue fluorescent compounds. This fraction was mixed with fraction B. *Fraction B* (250 mg) was chromatographed several times on Si gel. The eluents were C_6H_6–EtOAc mixtures and CH_2Cl_2–CCl_4 (2:1) to which increasing amounts of EtOAc were added. In addition to columbianadin (**3**), selinidin (**4**) (86 mg) was obtained. Mp 94.5–96.0, $[\alpha]_D^{26}$ − 18.0 ($CHCl_3$; *c* 0.8) (lit. [8] mp 97–98°, lit. [9] $[\alpha]_D^{25}$ − 17.8). The ^{1}H NMR and IR spectra were concordant with those of an authentic sample. *Fraction C* (*ca* 1 g) TLC on Si gel using CCl_4–MeOH (8:2) as the eluent divided fraction C into 3 blue fluorescent spots. However, all attempts to separate this mixture by column chromatography (Si gel or acetylated cellulose, 20%) or by PLC were unsuccessful. Only mixtures of at least three different compounds were obtained. ^{1}H NMR spectra of fraction C and of the fractions obtained in the attempts to separate fraction C disclosed that all fractions were mixtures of monoesters of khellactone (**7**), the acid moieties being angelic, senecioic, and isovaleric acid. *Fraction D* (1.8 g) was chromatographed on acetylated cellulose (Machery–Nagel 2100 AC/*ca* 20%). The eluent was MeCOEt–Me_2CO–H_2O (1:3:5) and the sample/column packing ratio 1:150. Hereby fraction D was divided into two fractions D_1 and D_2. D_1 was chromatographed on Si gel impregnated with 3% of H_2O using CH_2Cl_2–CCl_4 (2:1) to which increasing amounts of EtOAc were added as the eluent. The following compounds were obtained: a. Lomatin (**5**) (18 mg): mp 182–183°, $[\alpha]_D^{24}$ + 50° (EtOH; *c* 0.2) (lit. [5] mp 182.5–183.5°, $[\alpha]_D^{23}$ + 52°). The IR and ^{1}H NMR spectra were identical with those of an authentic sample. b. (+)-dihydrooroselol (**6**) (14 mg): mp 162.5–163.5°, $[\alpha]_D^{23}$ + 264° (MeOH: *c* 0.2) (lit. [5] mp 163.5–164.0°, $[\alpha]_D^{20}$ + 273°). The IR and ^{1}H NMR spectra were identical with those of an authentic sample. D_2 was chromatographed in the same manner as described for D_1 followed by rechromatography of appropriate fractions on Si gel using CCl_4–MeOH (97:3) as the eluent. The following substances were obtained: *c. trans-p*-coumaroyllomatin (**1**) (226 mg): a colourless glassy substance. $[\alpha]_D^{25.8}$ + 370° ($CHCl_3$; *c* 0.7). λ_{max}^{EtOH} nm (log ϵ) 318 (4.54), 302 (*sh*) (4.43). ^{1}H NMR (60 M Hz, $CDCl_3$; δ values; internal standard TMS): 1. The coumarin moiety: 6.18 (1 H, *d*, $J_{3,4}$ = 9.5 Hz, C-3), 7.62 (1 H, *d*, $J_{3,4}$ = 9.5 Hz, C-4), 7.25 (1 H, *d*, $J_{5,6}$ = 8.5 Hz, C-5), 6.78 (1 H, *d*, $J_{5,6}$ = 8.5 Hz, C-6), 5.0–5.3 (1 H, slightly broadened *t*, C-3′), 2.9–3.2 (2 H, *m*, C-4′), 1.35 and 1.33 (6 H, two *s*, *gem* dimethyl protons). 2. The acid moiety: 6.15 (1 H, *d*, J_{ab} = 16 Hz, H_a), 7.60 (1 H, *d*, J_{ab} = 16 Hz, H_b), 7.35 and 6.82 (4 H, two *d*, *J* = 9 Hz, H_c), 6.50 (1 H, broad *s*, H_d). The signal at 6.50 disappeared on deuteration. d. *cis-p*-coumaroyllomatin (**2**) (16 mg), an amorphous powder, melting range 171.5–176.0°, $[\alpha]_D^{25.3}$ − 12° ($CHCl_3$; *c* 1.0); λ_{max}^{EtOH} nm (log ϵ) 318 (4.50), 302 (*sh*) (4.41). ^{1}H NMR (60 M Hz, $CDCl_3$, δ values with reference to TMS): 1. The coumarin moiety: 6.20 (1 H, *d*, $J_{3,4}$ = 9.5 Hz, C-3), 7.62 (1 H, *d*, $J_{3,4}$ = 9.5 Hz, C-4), 7.23 (1 H, *d*, $J_{5,6}$ = 8.5 Hz, C-5), 6.72 (1 H, *d*, $J_{5,6}$ = 8.5 Hz, C-6), 5.0–5.3 (1 H, slightly broadened *t*, C-3′), 2.8–3.1 (2 H, *m*, C-4′), 1.31 and 1.28 (6H, two *s*, *gem* dimethyl protons). 2. The acid moiety: 5.73 (1 H, *d*, J_{ab} = 13 Hz, H_a), 6.85 (1 H, *d*, J_{ab} = 13 Hz, H_b), 7.50 and 6.77 (4 H, two *d*, *J* = 9 Hz, H_c), 6.6 (1 H, broad *s*, H_d). The signal at 6.6 disappeared on deuteration. Comparison of the ^{1}H NMR spectrum of (**2**) with that of *cis-p*-coumaric acid methylester confirmed the interpretation of the spectrum of (**2**) with respect to the acid moiety.

Saponification of (**1**). 31 mg of (**1**) were saponified by treatment with 0.5 N methanolic NaOH at 50° for 7 hr and worked up as previously described [10]. The yield of lomatin (**5**) was 15 mg, mp 183.5–184.5°, $[\alpha]_D^{22.2}$ + 53.7° (EtOH; *c* 0.2) (lit. [5], mp 182.5–183.5, $[\alpha]_D^{23}$ + 52°). The IR spectrum was identical with that of an authentic sample. In addition 4 mg of *trans-p*-coumaric acid, mp 207.5–209.5° (lit. [11] mp 210–213°) were obtained. The IR spectrum of the acid isolated was identical with that of an authentic sample.

Saponification of **2**. 15 mg of (**2**) were saponified with 0.5 N methanolic NaOH at 50° for 21 hr as described above. The yield of lomatin (**5**) was 5 mg: mp 182.5–183.5°, $[\alpha]_D^{23.7}$ + 50.6° (EtOH; *c* 0.3). The identity was established by IR spectroscopy. The acid phase upon chromatography on Si gel (1 g) using CH_2Cl_2–CCl_4–MeOH–HCOOH (120:70:10:1) as the eluent afforded 1 mg of a crystalline material with a melting range 125–195° (lit. [12] *cis-p*-coumaric acid mp 126–127°). IR spectroscopy of this material showed it to be a mixture of *cis*- and *trans-p*-coumaric acid.

Synthesis of cis-p-*coumaric acid.* The synthesis was carried out in analogy with the method used by Bregman *et al.* [13] in their synthesis of *p*-methoxy-*cis*-cinnamic acid from the *trans* isomer. 0.3 g of *trans-p*-coumaric acid were dissolved in 0.5% aqueous Na_2CO_3. The soln was deaerated with a stream of N_2 and subsequently irradiated for 200 min with a high pressure 125 W Hg immersion lamp. The reaction mixture was acidified with 4N HCl and extracted with Et_2O. Chromatography on Si gel (25 g) using CH_2Cl_2–CCl_4–MeOH–HCOOH (120:70:10:1) as the eluent afforded 80 mg of *cis-p*-coumaric acid, mp 127–128° recrystallized from C_6H_6)

(lit. [12] mp 126–127°). The compound gave satisfactory IR and ^1H NMR spectra.

Methyl esters of cis- *and* trans-p-*coumaric acid.* The esters were prepared in the usual manner using CH_2N_2. *Cis-p*-coumaric acid methylester mp 85.5–86° (Not described in lit.); *trans-p*-coumaric acid methylester, mp 136.5–137° (lit. [14] mp 136–137°).

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