

Constituents of Umbelliferous Plants

XVII.* Coumarins from *Seseli gummiferum* Pall. The Structure of Two New Coumarins

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From the stems of *Seseli gummiferum* Pall. subsp. *gummiferum* two new coumarins were isolated. The coumarins (I), $C_{24}H_{28}O_7$, and (II), $C_{24}H_{26}O_7$, are shown to be 3'(R),4'(R)-3'-isovaleryloxy-4'-angeloyloxy-3',4'-dihydroseselin and 3'(R),4'(R)-3'-seneciolyoxy-4'-angeloyloxy-3',4'-dihydroseselin, respectively.

Furthermore, the stems afforded 3'(R),4'(R)-3'-acetoxy-4'-seneciolyoxy-3',4'-dihydroseselin (III), (+)-*cis*-khellactone (V), and a mixture of monoesters of *cis*-khellactone.

In a previous paper,¹ the isolation and structure elucidations of three new lomatin esters from the stems of *Seseli gummiferum* Pall. subsp. *gummiferum* were reported. This paper presents the results from an investigation of the more polar coumarins.

Fractionation on silica gel columns and ¹H NMR-spectroscopic examination of the fractions disclosed that stems of *Seseli gummiferum* Pall. subsp. *gummiferum* contain a very complex mixture of diesters derived from *cis*-khellactone as well as a complex mixture of monoesters of *cis*-khellactone. The latter mixture constitutes the major part of the coumarins.

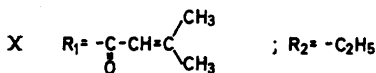
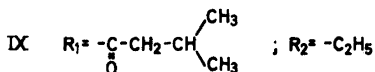
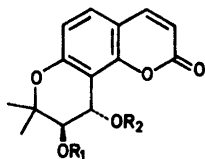
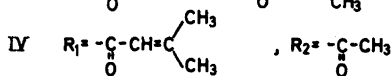
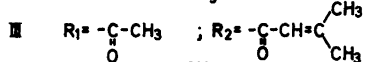
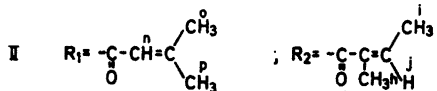
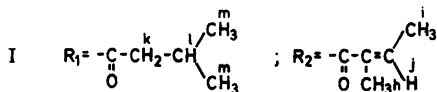
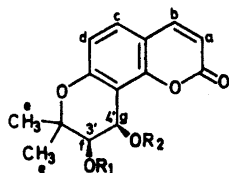
From the ¹H NMR-spectra of the diesters of *cis*-khellactone it appeared that the acid moieties were C₅-acids and acetic acid. Repeated chromatographic separations afforded, in addition to 3'(R),4'(R)-3'-acetoxy-4'-seneciolyoxy-3',4'-dihydroseselin² (III), only two pure khellactone diesters, (I), $C_{24}H_{28}O_7$, and (II), $C_{24}H_{26}O_7$. So far, (I) has not been obtained in a crystalline state.

3'(R),4'(R)-3'-Acetoxy-4'-seneciolyoxy-3',4'-dihydroseselin (III), first isolated from roots of *Seseli libanotis* (L.) Koch subsp. *eu-libanotis*,² was obtained in a crystalline modification (m.p. 106–108°) different from that (m.p. 121°) described by Lemmich *et al.*² An authentic sample of coumarin (III), kept at

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room temperature for several years, was, however, shown to be partly transformed into a modification with m.p. 106–108°. The two modifications of (III) were separated manually, and the identity of the lowest melting modification and (III) obtained from *Seseli gummiferum* Pall. subsp. *gummiferum* was established. The 3'(R),4'(R)-chirality of (III) has been established by Lemmich *et al.*³

From the ¹H NMR-spectra, compounds (I) and (II) appeared to be diesters of *cis*-khellactone with isovaleric and angelic acids, and with senecioic and angelic acids, respectively. The relative *cis*-configuration of both (I) and (II) is deduced from the observations of $J_{3',4'} = 5.0$ cps and of a slightly broadened singlet arising from the *gem*-dimethyl group.²



The relative positions of the acyl groups in the coumarins (I) and (II) were established in the usual way.⁴⁻⁶ Thus, treatment of (I) with ethanolic potassium hydroxide for a short time afforded angelic acid, a non-crystalline compound (IX), and small amounts of *cis*- and *trans*-ethylkhellactone (VI) and (VIII). The constitution of (IX) was established by ¹H NMR-spectroscopy, and from the observations of a $J_{3',4'} = 2.0$ cps and of well-separated *gem*-dimethyl signals (δ 1.39 and 1.46) it is quite safe to assign the relative *trans*-configuration to (IX)² bearing out the expectations based on chemical grounds. Finally, coumarin (I) was subjected to alkaline hydrolysis in dioxane to yield, besides angelic and isovaleric acids, *cis*- and (-)-*trans*-khellactone (V) and (VII).

(VII) is known to possess the 3'(R),4'(S)-chirality.³ Thus, based on spectral and chemical evidences, coumarin (I) is assigned the structure 3'(R), 4'(R)-3'-isovaleryloxy-4'-angeloyloxy-3',4'-dihydroseselin.

Coumarin (II), upon treatment with ethanolic potassium hydroxide, afforded angelic acid, the non-crystalline coumarin (X), and small amounts of the epimeric compounds (VI) and (VIII). Smith *et al.*,⁴ by a similar treatment of samidin (IV), obtained (X) in a crystalline state. All attempts to crystallize (X) were unsuccessful. Saponification of coumarin (X) with ethanolic potassium hydroxide provided senecioic acid and (-)-*trans*-ethylkhellactone (VIII), which is known as a degradation product from diesters of (+)-*cis*-khellactone (V)^{4,5} possessing the 3'(R),4'(R)-chirality. Conclusive evidence is now provided for the structure of (II) as 3'(R),4'(R)-3'-senecioyloxy-4'-angeloyloxy-3',4'-dihydroseselin.

As mentioned above, the main part of the coumarins was a mixture of monoesters of *cis*-khellactone, the acid moieties being angelic, senecioic, and isovaleric acids. So far, all attempts to isolate the individual coumarins in a pure state have been unsuccessful.

Finally, the stems afforded the well known coumarin (+)-*cis*-khellactone (V). To our knowledge, however, (+)-*cis*-khellactone has not previously been obtained from natural sources.

EXPERIMENTAL

Melting points, determined in capillary tubes, are corrected. IR-spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer, Model 457. The spectra of solids were recorded in potassium bromide discs and those of non-crystalline compounds in chloroform solutions. ¹H NMR-spectra (CCl₄) were measured on a JEOL JNM-C-60HL instrument. Thin-layer chromatography was carried out, using silica gel GF₂₅₄ as adsorbent. Silica gel, used in column chromatography, was treated as earlier described.⁷ Microanalyses were performed by Dr. A. Bernhardt, Elbach über Engelskirchen, West Germany.

Gross fractionation of the coumarin mixture. Column chromatography of a defatted extract (20 g), obtained from the dried stems (800 g), has been described in an earlier paper.¹ Further elution of the column with methylene chloride-tetrachloromethane (2:1), to which ethyl acetate and methanol were added in the concentrations mentioned below, yielded the following fractions:

- fraction B* (4.2 g) (3.5–4.5 % of ethyl acetate),
- fraction C* (1.7 g) (4.5–6.5 % of ethyl acetate),
- fraction D* (0.78 g) (6.5–8.0 % of ethyl acetate),
- fraction E* (7.8 g) (8.0–20 % of ethyl acetate), and
- fraction F* (0.17 g) (20 % of ethyl acetate – 22 % of ethyl acetate and 2 % of methanol).

Isolation of (+)-cis-3'-isovaleryloxy-4'-angeloyloxy-3',4'-dihydroseselin (I). Chromatography of fraction B (4.2 g) and rechromatography of appropriate fractions several times on silica gel, using benzene-ethyl acetate mixtures as eluents, afforded 375 mg of coumarin (I) as a colourless glassy substance, $[\alpha]_D^{24,5} + 21.4^\circ$ (c 0.8, CHCl₃). (Found: C 67.10; H 6.47. Calc. for C₂₄H₂₈O₇: C 67.27; H 6.59.) The IR- and ¹H NMR-spectra were consistent with the constitution and relative configuration assigned to (I).

¹H NMR-data (δ -values). Doublets at 6.15 (a) and at 7.63 (b), 1H each, $J_{a,b} = 9.5$ cps. Doublets at 7.43 (c) and at 6.78 (d), 1H each, $J_{c,d} = 8.7$ cps. Doublets at 6.46 (g) and at 5.24 (f), 1H each, $J_{g,f} = 5.0$ cps. Slightly broadened peak at 1.42, 6H (e). The following ¹H NMR-data originate in the acid moieties of coumarin (I): a complex pattern at ca. 5.7–6.2, 1H (j); multiplets at ca. 1.7–2.0, 6H (h and i); perturbed doublets at ca. 2.0–2.3, 2H (k) and at ca. 0.8–1.1, 6H (m).

Treatment of (I) with ethanolic potassium hydroxide. A solution of coumarin (I) (109 mg) in 6 ml of ethanol was mixed with 7 ml of 1 N ethanolic potassium hydroxide and left at room temperature for 6 min. The solution was acidified with 2 N sulfuric acid (7 ml) and diluted with 18 ml of water. Upon standing for 45 min, the reaction mixture was adjusted to pH 8 with sodium carbonate solution and extracted with chloroform. The extract, after drying and evaporation, was chromatographed on silica gel (26 g). As eluent was used tetrachloromethane-methylene chloride (2:1), to which increasing amounts of ethyl acetate were added. Obtained were:

a. 53 mg of *trans*-3'-isovaleryloxy-4'-ethoxy-3',4'-dihydroseselin (IX) as a colourless uncrystallizable oil. The constitution and relative configuration assigned to (IX) were consistent with the IR- and ^1H NMR-spectra.

b. *Ca.* 1 mg of *cis*-ethylkhellactone (VI) which was identified by co-chromatography with an authentic sample.

c. 6.5 mg of *trans*-ethylkhellactone (VIII), m.p. 160.0–160.5° (ether-petroleum ether) (Ref. 4, m.p. 161–162°). The IR-spectrum was identical with that of an authentic sample.

The aqueous phase was acidified and extracted with ether. The ether phase was filtered and dried. Upon addition of 100 μl of dicyclohexylethylamine, the solvent was evaporated. The *p*-phenylphenacyl ester was prepared according to Stodola⁸ and isolated by chromatography on silica gel (10 g) using petroleum ether-benzene mixtures as eluents. Besides unreacted *p*-phenylphenacyl bromide, 31 mg of *p*-phenylphenacyl angelate, m.p. 88.0–89.0° (ethanol-water), were obtained. The IR-spectrum was identical with that of an authentic sample.

Treatment of (I) with 1 N potassium hydroxide. A solution of coumarin (I) (100 mg) in 1 ml of dioxane and 3 ml of 1 N potassium hydroxide was left at room temperature for 15 h. The reaction mixture was worked up as described above. The chloroform phase was chromatographed on silica gel (26 g), using methylene chloride-ethyl acetate (4:1), to which increasing amounts of methanol were added, as eluent. Obtained were:

a. 6.5 mg of *cis*-khellactone, m.p. 174.0–174.5° (methylene chloride-tetrachloromethane) (Ref. 9, m.p. 174–175°). The IR-spectrum was identical with that of an authentic sample.

b. 22.5 mg of (-)-*trans*-khellactone (VII), m.p. 187.0–188.0° (chloroform-benzene), $[\alpha]_{\text{D}}^{21.8} - 19.5^\circ$ (c 0.6, CHCl_3) (Ref. 9, m.p. 185–186°, $[\alpha]_{\text{D}}^{21} - 18^\circ$). The IR-spectrum was identical with that of an authentic sample.

Workup of the aqueous phase afforded:

c. 18 mg of *p*-phenylphenacyl angelate, m.p. 86.5–88.0° (methanol-water). The identity was established by IR-spectroscopy.

d. 20 mg of *p*-phenylphenacyl isovalerate, m.p. 77.0–78.5° (methanol-water). The identity was established by IR-spectroscopy.

Isolation of (+)-cis-3'-seneciolyloxy-4'-angeloyloxy-3',4'-dihydroseselin (II). Chromatography of fraction C (1.7 g) and rechromatography of appropriate fractions several times on silica gel, using petroleum ether-ethyl acetate mixtures as eluents, afforded 86 mg of coumarin (II), m.p. 78.0–79.5° (ether-petroleum ether), $[\alpha]_{\text{D}}^{21.8} + 17.9^\circ$ (c 0.9, CHCl_3). (Found: C 67.73; H 6.32. Calc. for $\text{C}_{24}\text{H}_{26}\text{O}_7$: C 67.59; H 6.15.) The IR- and ^1H NMR-spectra were consistent with the constitution and relative configuration assigned to (II).

^1H NMR-data (δ -values). Concerning the protons *a*–*j*, see above for coumarin (I). Multiplets at ca. 5.5–5.6, 1H (*n*); at ca. 2.1–2.2, 3H (*p*); and at ca. 1.8–1.9, 3H (*o*).

Treatment of (II) with ethanolic potassium hydroxide. A solution of coumarin (II) (71 mg) in 4 ml of ethanol was treated with 5 ml of 1 N ethanolic potassium hydroxide, and the reaction mixture was worked up as described above for coumarin (I). Obtained were:

a. 38 mg of (+)-*trans*-3'-seneciolyloxy-4'-ethoxy-3',4'-dihydroseselin (X) as a colourless oil, $[\alpha]_{\text{D}}^{20.0} + 95^\circ$ (c 0.9, 95% ethanol) (Ref. 4, m.p. 91–93°, $[\alpha]_{\text{D}} + 99^\circ$ (c 0.9)). The ^1H NMR-spectrum was consistent with the constitution and relative configuration assigned to (X).

b. Trace amounts of *cis*-ethylkhellactone, which was identified by co-chromatography with an authentic sample.

c. 3.0 mg of *trans*-ethylkhellactone, m.p. 159.0–160.5° (ether-petroleum ether). The IR-spectrum was identical with that of an authentic sample.

d. 11 mg of *p*-phenylphenacyl angelate, m.p. 88.5–89.5° (ethanol-water). The identity was established by IR-spectroscopy.

Ethanolic saponification of (X). A solution of compound (X) (36 mg) in 3 ml of 1 N ethanolic potassium hydroxide was left at room temperature for 20 h. Workup of the product in the usual way afforded:

a. 15 mg of (–)-*trans*-ethylkhellactone (VIII), m.p. 161.0–161.5° (ether-petroleum ether), $[\alpha]_D^{20.0} - 59^\circ$ (c 0.9, 95 % ethanol) (Ref. 4, m.p. 161–162°, $[\alpha]_D - 59^\circ$ (c 0.9)). The IR-spectrum was identical with that of an authentic sample.

b. 8 mg of *p*-phenylphenacyl senecioate, m.p. 141–142° (ethanol-water). The identity was established by IR-spectroscopy.

Isolation and identification of (–)-cis-3'-acetoxy-4'-seneciolyloxy-3',4'-dihydroseselin (III). Fraction D (0.78 g) was subjected to chromatography and appropriate fractions to rechromatography several times on silica gel, using benzene-ethyl acetate mixtures as eluents, and was finally separated into two fractions.

a. 145 mg of a non-crystalline mixture of diesters of *cis*-khellactone, which was homogenous on TLC-plates in several solvent systems. The acid moieties were acetic, angelic, isovaleric, and senecioic acids.

b. 310 mg of a crystalline fraction, which by ¹H NMR-spectroscopy was shown to be a mixed diester of *cis*-khellactone (V) with acetic acid and senecioic acid. Several fractionated crystallizations, using ether-petroleum ether, yielded 85 mg of coumarin (III), m.p. 106–108°, $[\alpha]_D^{24.9} - 11.6^\circ$ (c 0.4, C₂H₅OH) (Ref. 2, m.p. 120.5–121°, $[\alpha]_D^{25} - 11.9^\circ$ (c 0.4, C₂H₅OH)). The IR-spectrum was identical with that of a modification of authentic (III) with m.p. 106–108°.

Mixture of monoesters of cis-khellactone. The non-crystalline fraction E (7.8 g) was by ¹H NMR-spectroscopy shown to be a mixture of monoesters of *cis*-khellactone, the acid moieties of which were angelic, isovaleric, and senecioic acids in the ratios ca. 3:2:2. On TLC-plates, using tetrachloromethane-methanol (19:1) as eluent, fraction E was split up into three blue-fluorescent spots (*R_F* 0.37, 0.46, and 0.51). Several attempts to obtain the individual coumarins of this fraction in a pure state, by column and preparative thin-layer chromatography, were unsuccessful.

Isolation and identification of (+)-cis-khellactone (V). Chromatography of fraction F (0.17 g) on silica gel, using benzene-ethyl acetate (19:1), to which increasing amounts of methanol were added, as eluent, afforded 82 mg of (+)-*cis*-khellactone (V), m.p. 174.5–175.0° (methylene chloride-tetrachloromethane, $[\alpha]_D^{25.0} + 82.1^\circ$ (c 0.6, CHCl₃) (Ref. 9, m.p. 174–175°, $[\alpha]_D^{20} + 81^\circ$). The IR-spectrum was identical with that of an authentic sample.

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