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Constituents of Umbelliferous Plants

XIII.* Coumarins from *Seseli gummiferum* Pall. The Structure of Three New Coumarins

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Three coumarins, obtained from the stems of *Seseli gummiferum* Pall. subsp. *gummiferum* are shown to be 3′(*R*)-(+)-3′-hexanoyloxy-3′,4′-dihydroseselin (IV), 3′(*R*)-(+)-3′-octanoyloxy-3′,4′-dihydroseselin (V), and 3′(*R*)-(+)-3′-*cis*-4-octenoyloxy-3′,4′-dihydroseselin (VI).

Furthermore, the stems afforded deltoin (II).

In a previous paper[1] the determination of the absolute configuration of falcarinol, isolated from the roots of *Seseli gummiferum* Pall. subsp. *gummiferum*, has been reported. In addition the roots afforded a small amount of a rather complex mixture of coumarins. The stems of *Seseli gummiferum* Pall. subsp. *gummiferum*, which by incision exude a thick and sticky latex, afforded an about ten times higher yield of the same coumarin mixture. The qualitative identity of the two coumarin mixtures was established by comparative thin-layer chromatographic and ^{1}H-NMR-spectroscopic examinations of corresponding fractions obtained by column chromatography on silica gel. Only the mixture originating from the stems was worked up for final characterization and identification of the individual coumarins. In addition to deltoin (II) and three new coumarins (IV), (V), and (VI), a group of more polar coumarins, which is presently under investigation, were obtained.

Deltoin (II), which has been isolated for the first time by Nikonov,[2] was identified by m.p. determination, IR-, and ^{1}H-NMR-spectroscopy. Deltoin (II) is the angelate of marmesin (I), the configuration of which has recently been shown to be (*S*).[3]

All attempts to crystallize the coumarins (IV), (V), and (VI) were unsuccessfull. From the UV-, IR-, and ^{1}H-NMR-spectra, (IV), (V), and (VI) appeared

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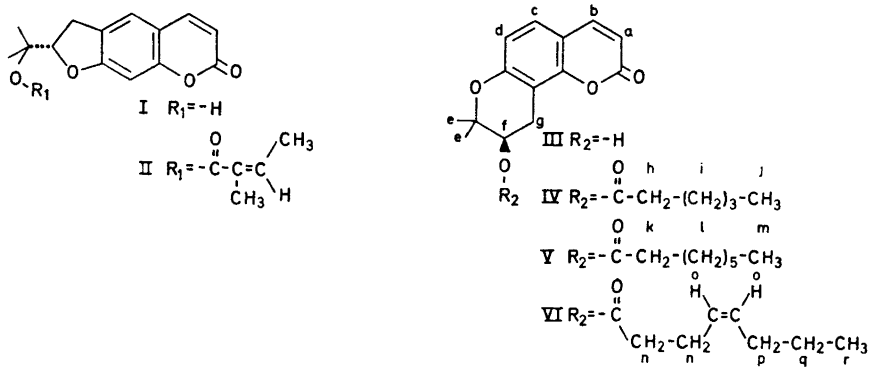
to be esters of lomatin (III) with fatty acids. Usually the coumarins are esterified with C_5-acids or acetic acid and so these findings apparently break this pattern. The constitutions of (IV), (V), and (VI) have been established by mass spectrometry. The mass spectra are consistent with the fragmentations proposed by Shipchandler and Soine [4] for lomatin (III) and some of its derivatives.

Furthermore, saponification of (IV), (V), and (VI) afforded lomatin (III) and, in addition, hexanoic, octanoic, and *cis*-4-octenoic acid, respectively. The two former acids were identified through formation of their *p*-phenylphenacyl esters. The position of the double bond in the octenoic acid was established by ^{1}H-NMR-spectroscopy and by oxidation with sodium periodate and potassium permanganate. The oxidation products were identified after conversion to their *p*-bromophenacyl esters. Support for the *cis*-configuration of 4-octenoic acid was derived from the work carried out by Knight and Diamond,[5] who have investigated the IR-spectra of several isomeric octenoic acids, including *cis*-4-octenoic acid. It was shown by these authors that the *trans*-isomers, unlike the *cis*-isomers, exhibit strong absorption bands in the region of 10.2 – 10.35 μ. These bands were missing in the IR-spectrum of 4-octenoic acid from (VI). Finally *cis*-4-octenoic acid was characterized through formation of its *p*-bromophenacyl ester. The constitutions of (IV) and (V) were confirmed by synthesis.

The absolute configuration of lomatin (III) has recently been shown to be (*R*) [6] and accordingly the coumarins (IV), (V), and (VI) are 3′(*R*)-(+)-3′-hexanoyloxy-3′,4′-dihydroseselin (IV), 3′(*R*)-(+)-3′-octanoyloxy-3′,4′-dihydroseselin (V), and 3′(*R*)-(+)-3′-*cis*-4-octenoyloxy-3′,4′-dihydroseselin (VI), respectively.

To our knowledge *cis*-4-octenoic acid or esters derived from it have not been isolated from natural sources before.



EXPERIMENTAL

Melting points and ^{1}H-NMR-spectra (CCl_4) were determined as in a previous paper.[7] IR-spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer, Model 457. Spectra of solids were recorded as earlier described.[7] IR-spectra of liquids were

measured in tetrachloromethane solutions. UV-spectra were recorded in ethanol. Mass spectra were measured on an MS 902 mass spectrometer at 70 eV. Thin-layer chromatography was carried out, using silica gel GF_{254} as the adsorbent. Silica gel, used in column chromatography, was treated as earlier described,[1] unless otherwise is stated. Microanalyses were performed by Dr. A. Bernhardt, Elbach über Engelskirchen.

Isolation and gross fractionation of the coumarin mixture. The dried and ground stems (800 g) on extraction with ether and subsequent evaporation of the solvent afforded 31 g of an oily residue, which was dissolved in 90 % methanol and freed of lipids and the bulk of chlorophylls by extraction with petroleum ether. The defatted extract (20 g) was chromatographed on silica gel (450 g). The eluent was methylene chloride-tetrachloromethane (2:1) to which increasing amounts of ethyl acetate were added. With 2.5—3.5 % of ethyl acetate a mixture of the coumarins (II), (IV), (V), and (VI) (fraction A) (1.0 g) was eluted.

Isolation and identification of deltoin (II). Chromatography of fraction A on silica gel (100 g), using benzene-ethyl acetate mixtures as the eluents, afforded 18 mg of impure (II), and 910 mg of a mixture of (IV), (V), and (VI) (fraction B). Rechromatography of (II) on silica gel (10 g), using benzene as the eluent, afforded 11 mg of deltoin (II), m.p. 105.0—105.5° (ether-petroleum ether), $[\alpha]_D^{16.1}$ −38.5° (*c* 0.8, $CHCl_3$) (Ref. 2, m.p. 105—106.5°, $[\alpha]_D^{16}$ −51.2° (*c* 1.0, $CHCl_3$)).

The results of the elementary analysis agreed with the theoretical values. The ^{1}H-NMR-spectrum confirmed the constitution of (II). The IR-spectrum agreed with that published by Nikonov [2] for deltoin (II).

Isolation of (+)-hexanoyllomatin (IV). Chromatography of fraction B and rechromatography of appropriate fractions several times on silica gel, using benzene-ethyl acetate mixtures as the eluents, afforded 49 mg of compound (IV) as a colourless oil and 601 mg of a mixture of (V) and (VI) (fraction C). Thin-layer chromatography and mass spectrometry of (IV) demonstrated that (IV) was obtained in a pure state. $[\alpha]_D^{20.1}$ +30.1° (*c* 1.1, CCl_4). IR-, UV-, and ^{1}H-NMR-spectra were consistent with the constitution assigned to (IV).

^{1}H-NMR-data (δ-values): Doublets at 6.08 (*a*) and 7.54 (*b*), 1 H each, J=9.5 cps. Doublets at 7.19 (*c*) and 6.66 (*d*), 1 H each, J=8.5 cps. Slightly broadened triplet at *ca.* 5.0—5.3, 1 H (*f*). Complex pattern at *ca.* 2.6—3.4, 2 H (*g*). A slightly broadened singlet at 1.33, 6 H (*e*). The following ^{1}H-NMR-data originate in the acid moiety of coumarin (IV): A perturbed triplet at *ca.* 2.1—2.4, 2 H (*h*). Multiplets at *ca.* 1.0—1.7, 6 H (*i*) and at *ca.* 0.7—1.0, 3 H (*j*).

Prominent peaks in the mass spectrum of (IV) (*m/e*): 344 (M^+, 10 %), 229 (12 %), 228 (29 %), 214 (13 %), 213 (100 %), 187 (8 %), 176 (20 %), 175 (9 %), 131 (6 %), 99 (13 %), 71 (14 %), 43 (34 %), and 41 (8 %).

Separation and isolation of (+)-octanoyllomatin (V) and (+)-cis-4-octenoyllomatin (VI). Judging from thin-layer chromatography, using several solvent systems, fraction C appeared to be homogeneous. Mass spectrometry, however, showed it to be a mixture of two compounds with molecular peaks at *m/e* 372 and *m/e* 370 and with fragmentation patterns very similar to that of compound (IV). Fraction C was rechromatographed on TLC-plates, using silica gel impregnated with 3.3 % of silver nitrate and 3.3 % of boric acid as the adsorbent.[8] As the eluent was used methylene chloride-ethyl acetate (9:1). Under these conditions fraction C was separated into two blue-fluorescent spots (R_F 0.45 and 0.67). Fraction C was chromatographed on silica gel (60 g), which prior to use was mixed with a solution of 3 g of silver nitrate in 100 ml of 95 % methanol, evaporated to dryness, activated at 150° for 15 min and impregnated with 5 % of water. As the eluent was used methylene chloride to which increasing amounts of ethyl acetate were added. The compounds (V) and (VI) were easily separated and isolated in pure state. Both substances were, by mass spectrometry, shown to be free from homologs. Obtained were:

a. 503 mg of (+)-octanoyllomatin (V) as a colourless oil, $[\alpha]_D^{20.1}$ +32.5° (*c* 1.0, CCl_4). (Found: C 71.06; H 7.64. Calc. for $C_{22}H_{28}O_5$: C 70.94; H 7.58). The constitution assigned to (V) was consistent with the IR-, UV-, and ^{1}H-NMR-spectra.

^{1}H-NMR-data (δ-values): Concerning the protons *a*—*g*, see above for coumarin (IV). A perturbed triplet at *ca.* 2.1—2.4, 2 H (*k*). Multiplets at *ca.* 1.0—1.7, 9 H (*l*) and at *ca.* 0.7—1.0, 3 H (*m*).

Prominent peaks in the mass spectrum of (V) (*m/e*): 372 (M^+, 12 %), 229 (15 %), 228 (42 %), 214 (16 %), 213 (100 %), 187 (8 %), 176 (19 %), 175 (9 %), 127 (6 %), 71 (6 %), 57 (25 %), 43 (18 %), and 41 (7 %).

b. 64 mg of (+)-*cis*-4-octenoyllomatin (VI) as a colourless oil, $[\alpha]_D^{20.1}$ +30.7° (*c* 1.0, CCl_4). The IR-, UV-, and ^{1}H-NMR-spectra exhibited the absorption bands expected for compound (VI).

^{1}H-NMR-data (δ-values): As to the protons *a*—*g*, see above for coumarin (IV). A perturbed triplet at *ca.* 5.2—5.4, 2 H (*o*). Multiplet at *ca.* 2.2—2.4, 4 H (*n*). A broadened triplet at *ca.* 1.8—2.2, 2 H (*p*). Multiplets at *ca.* 1.1—1.6, 2 H (*q*) and at *ca.* 0.7—1.0, 3 H (*r*).

Prominent peaks in the mass spectrum of (VI) (*m/e*): 370 (M^+, 17 %), 229 (18 %), 228 (39 %), 214 (16 %), 213 (100 %), 187 (9 %), 176 (20 %), 175 (9 %), 125 (3 %), 97 (4 %), 83 (5 %), 71 (6 %), 55 (31 %), 43 (8 %), and 41 (9 %).

Saponification of (IV). 38 mg of (IV) were disolved in 3 ml of 1 N methanolic potassium hydroxide and left overnight at room temperature. The solution was diluted with water (10 ml) and acidified with 4 N sulfuric acid, finally adjusted to pH 8 with sodium carbonate solution and extracted with methylene chloride. The filtered and dried extract on evaporation afforded 15.5 mg of lomatin (III), m.p. 183.5—184.5° (methylenechloride-ether-petroleum ether), $[\alpha]_D^{23.1}$ +53.4° (*c* 0.8, C_2H_5OH) (Ref. 9, m.p. 187—188°, $[\alpha]_D^{23}$ +74.8° (C_2H_5OH); Ref. 6, m.p. 182.5—183.5°, $[\alpha]_D^{22}$ +52° (*c* 0.4, C_2H_5OH)). 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 Stodola [10] and isolated by chromatography on silica gel (11 g) using petroleum ether-benzene mixtures as the eluents. Besides unreacted *p*-phenylphenacyl bromide, 9.5 mg of *p*-phenylphenacyl hexanoate, m.p. 64.5—65.5° (ethanol-water) were obtained (Ref. 11, m.p. 64.2—65.4°). The IR-spectrum was identical with that of an authentic sample (m.p. 65.0—66.0°).

Saponification of (V). 100 mg of (V) were saponified, using 5 ml of 1 N methanolic potassium hydroxide, and the acid moiety of (V) converted to the *p*-phenylphenacyl ester, using the methods described above for compound (IV). Isolated were 50 mg of lomatin (III), m.p. 184.0—185.0° (methylene chloride-ether-petroleum ether), $[\alpha]_D^{23.0}$ +53.4° (*c* 0.8, C_2H_5OH). The identity was established by IR-spectroscopy.

The yield of *p*-phenylphenacyl octanoate was 30 mg with m.p. 67.5—68.5° (ethanol-water) (Ref. 11, m.p. 66—67°). The IR-spectrum was identical with that of an authentic sample (m.p. 67.5—68.5°).

Saponification of (VI). 55 mg of (VI) were saponified as described above for compound (IV). The yield of lomatin (III) was 23.5 mg, m.p. 183.5—184.5° (methylene chloride-ether-petroleum ether), $[\alpha]_D^{23.0}$ +52.9° (*c* 0.9, C_2H_5OH). The identity was established by IR-spectroscopy.

The aqueous phase was worked up in the usual way and afforded 15.5 mg of *cis*-4-octenoic acid as an oil. 10 mg of the acid were converted to the *p*-bromophenacyl ester according to Stodola.[10] Crystallization from methanol-water yielded 16 mg of *p*-bromophenacyl *cis*-4-octenoate, m.p. 48.0—49.0°. The melt crystallized at room temperature to a modification with m.p. 43.0—43.5° (Ref. 12, m.p. of *p*-bromophenacyl *cis*-4-octenoate, 41—42°. M.p. of *p*-bromophenacyl *trans*-4-octenoate, 78—79°). The IR-spectrum of *cis*-4-octenoic acid and the IR- and ^{1}H-NMR-spectra of *p*-bromophenacyl *cis*-4-octenoate were consistent with the assigned constitution and configuration.

Oxidative cleavage of cis-*4-octenoic acid*. 2.1 mg of *cis*-4-octenoic acid (0.0148 mmol) were oxidized with potassium permanganate-sodium periodate as described for methyl 9-decenoate by Grimmer and Jacob.[13] The evaporated and acidified reaction mixture, however, was mixed with diatomaceous earth-anhydrous sodium sulfate (3:1) (2 g), packed into a column and eluted with ether. The eluate was evaporated and the residue converted to a mixture of *p*-bromophenacyl esters according to Stodola.[10] The reaction mixture was chromatographed on silica gel (26 g), using methylene chloride-ethyl acetate mixtures as the eluents. Besides unreacted *p*-bromophenacyl bromide and small amounts of *p*-bromoacetophenone as a by-product (identified by TLC and mass spectrometry) the following compounds were isolated:

a. *p*-bromophenacyl succinate (2 mg), m.p. 212—213° (glacial acetic acid) (Ref. 14, m.p. 211°). The identity was established by IR-spectroscopy.

b. *p*-bromophenacyl butyrate (*ca*. 0.1 mg), identified by co-chromatography with an authentic sample in several solvent systems.

Preparation of (IV). To a solution of 100 mg of lomatin (III) (m.p. 183.5—184.5°) in 1.5 ml of anhydrous pyridine were added 400 µl of hexanoyl chloride. The mixture was left at room temperature for 65 h and was then worked up in the usual way. Chromatography on silica gel (30 g), using benzene-ethyl acetate mixtures as the eluents, afforded 86 mg of pure (IV) as a colourless oil. The IR-, UV-, and ^{1}H-NMR-spectra were identical with those of natural (IV).

Preparation of (V). Compound (V) was synthesized and isolated as described above for (IV). As the starting materials were used lomatin (III) (100 mg, m.p. 183.5—184.5°) and octanoyl chloride (400 µl). 105 mg of pure (V) were obtained as an oil. The IR-, UV-, and ^{1}H-NMR-spectra were identical with those of natural (V).

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