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Constituents of Umbelliferous Plants

VIII.* Coumarins from the Root of *Seseli libanotis* (L.) Koch. The Structure of Three New Coumarins

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The ether extract of the root of *Seseli libanotis* (L.) Koch subsp. *eu-libanotis*, in addition to the coumarins xanthotoxin, bergapten, psoralen, and pteryxin (I) afforded two new khellactone esters 3′(*S*),4′(*S*)-3′,4′-disenecioyloxy-3′,4′-dihydroseselin (II), and 3′(*S*),-4′(*S*)-3′-acetoxy-4′-senecioyloxy-3′,4′-dihydroseselin (III), and a new non-crystalline coumarin (IV), which is shown to be the angeloyl ester of (−)-3′-hydroxy-3′,4′-dihydroxanthyletin (V).

(±)-3′-Hydroxy-3′,4′-dihydroxanthyletin and (±)-4′-hydroxy-3′,4′-dihydroxanthyletin (VI) have been synthetized from natural xanthyletin (VII).

The application of NMR-spectroscopy in the assignment of relative configuration in khellactone derivatives is discussed.

Seseli libanotis (L.) Koch is a perennial herb, the typical form of which (subsp. *eu-libanotis* Thellung) is distributed over most of Europe.

The root of this plant upon extraction with ether afforded a rather complex mixture of coumarins. Fractionation on columns of silica gel afforded, in addition to small amounts of xanthotoxin, bergapten, and psoralen, two non-crystalline fractions, each of which consisted of closely related blue-fluorescent coumarins with similar chromatographic behaviour.

One of these fractions, which contained the major part of the coumarins, was shown to be a mixture of diesters derived from (+)-*cis*-khellactone (VIII), the acid moieties being acetic, isovaleric, 2-methylbutyric, senecioic, or angelic acids. Repeated chromatographic fractionations afforded, in addition to pteryxin (I), only two khellactone esters, (III), $C_{21}H_{22}O_7$, and (II), $C_{24}H_{26}O_7$, in a pure crystalline state.

Pteryxin (I), which has formerly been isolated from the root of *Pteryxia terebinthina* var. *terebinthina*,[1] was identified by comparison with an authentic sample.

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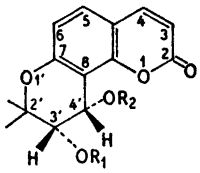
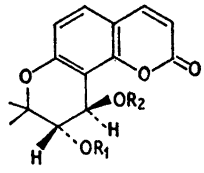
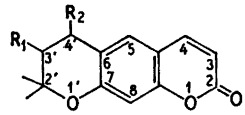
From the NMR-spectrum compound (II) appeared to be a khellactone disenecioate. Prolonged treatment of (II) with boiling methanolic hydrochloric acid afforded mainly (−)-*trans*-methylkhellactone (IX) and a small



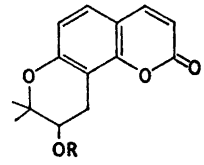
I R_1 = Acetyl ; R_2 = Angeloyl
II R_1 = R_2 = Senecioyl
III R_1 = Acetyl; R_2 = Senecioyl
VIII R_1 = R_2 = H
X R_1 = H; R_2 = CH_3
XIII R_1 = Senecioyl ; R_2 = Acetyl
XIV R_1 = 2-Methylbutyryl; R_2 = Acetyl
XV R_1 = Acetyl ; R_2 = Isovaleryl



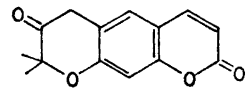
IX R_1 = H ; R_2 = CH_3
XI R_1 = R_2 = Senecioyl
XII R_1 = R_2 = H



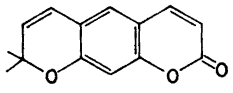
IV R_1 = Angeloyloxy; R_2 = H
V R_1 = OH; R_2 = H
VI R_1 = H; R_2 = OH
XXI R_1 = OH; R_2 = Benzoyloxy



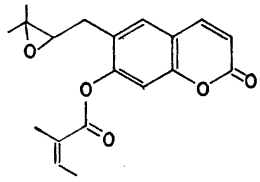
XVI R = Angeloyl
XVII R = H



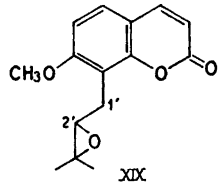
XX



VII



XVIII



XIX

amount of the corresponding *cis*-methylkhellactone (X), which were also obtainable in a similar way from a sample of the total khellactone ester fraction. The senecioic acid liberated from compound (II) was characterized as the *p*-phenylphenacyl ester. The formation of two epimeric ethers by methanolysis of (II) is a consequence of the S_N1 type reaction, which is known [1-3] to occur at the benzyl ester grouping at position 4′ in khellactone esters during their hydrolysis and methanolic or ethanolic solvolysis.

These epimerizations were first noted by Schroeder *et al.*,[3] who were also able to separate the epimeric khellactones and methylkhellactones and establish their absolute configurations. The methylkhellactones obtained from (II) are identical with those obtained from all hitherto known natural khellactone esters, thus establishing the configuration 3′(*S*) for compound (II). The configuration at the position 4′ was established by the synthesis of (II) and its epimer (XI) from (+)-*cis*-khellactone (VIII) and (−)-*trans*-khellactone (XII), respectively, by acylation with senecioic anhydride. On the basis of the configurational statements of Schroeder *et al.*[3] the structure 3′(*S*),4′(*S*)-3′,4′-disenecioyloxy-3′,4′-dihydroseselin is therefore assigned to compound (II).

From the NMR-spectrum, compound (III) (m.p. 121°) appeared to be a diester of *cis*-khellactone with acetic acid and senecioic acid and so apparently differed from samidin (XIII) (m.p. 139°), one of the constituents of the fruits of *Ammi visnaga*,[2,3] only by the interchanged positions of the acyl groups. The structure 3′(*S*),4′(*S*)-3′-acetoxy-4′-senecioyloxy-3′,4′-dihydroseselin for compound (III) was further proved by its conversion to suksdorfin (XV) by the hydrogenation of one double bond.

Most khellactone esters previously found in nature are mixed esters, the stereospecific syntheses of which have failed because of the facile epimerization at the position 4′. The *cis*-configuration for samidin (XIII) and visnadin (XIV)[3] and later for suksdorfin (XV) and pteryxin (I)[1] therefore had to be established by comparison of their IR-spectra with those of synthetic (±)-*cis*-diacetylkhellactone, (±)-*trans*-diacetylkhellactone and (±)-*trans*-samidin.

From the data compiled in Table 1 it appears, that also the NMR-spectra are useful for the assignment of relative configurations in khellactone derivatives and related compounds. Through the range of the *trans*-khellactone derivatives listed, the variation of the observed $J_{3',4'}$-values from 3.0 cps to 6.9 cps is accompanied by a variation from 0.08 ppm to 0.20 ppm of the difference between the chemical shifts observed for the two methyl groups in the chroman ring. For the *cis*-khellactone derivatives the same coupling constant is observed in all cases, and little or no difference is observed between the chemical shifts of the two methyl groups.

Half chair conformations or slightly distorted half chair conformations are the most likely for the chroman ring in khellactone derivatives, as has also been discussed for flavan derivatives (*vide*, *e.g.*, Refs. 4 and 5).

In *trans*-khellactone derivatives the dihedral angle between the protons at the positions 3′ and 4′, and accordingly their coupling constant, will differ distinctly, when going from one of the possible half chair forms to the other.

3,4-Flavandiols and their derivatives have been shown to be almost frozen conformers with the 2-aryl substituent equatorial.[5,6] 2,3-*trans*-3,4-*trans*-3,4-Disubstituted flavans ($J_{3,4}$ = 6.8—7.8 cps[7]) therefore appear to be suitable analogues to the conformer of *trans*-khellactone derivatives with two axial protons at the positions 3′ and 4′, and 2,3-*cis*-3,4-*trans*-3,4-disubstituted flavans ($J_{3,4}$ = 2.4—3.2 cps[7]) to the other conformer. The observed coupling constants correspond to time averages over the two possible conformations. The fact, that the $J_{3',4'}$-values found for various *trans*-khellactone derivatives are very different, therefore indicates great differences in conformational equilibria. For instance, the coupling constant $J_{3',4'}$ = 6.9 cps observed for *trans*-methylkhellactone, when compared with 3,4-disubstituted flavans appears to indicate the preponderance of the conformer with the 3′- and 4′-hydrogen atoms both axial. It is also interesting to note, from some of the rather low $J_{3',4'}$-values observed, that the spacial interaction between an axial methyl group at the position 2′ and a pseudoaxial oxygen substituent at the position 4′ is not serious.

It is likely that also the *cis*-khellactone derivatives listed in Table 1 are mutually different in conformational equilibria. The fact that the same $J_{3',4'}$-value is recorded for all these compounds is explained by the fact that the magnitude of the dihedral angle for the protons at the positions 3′ and 4′ is

Table 1. Some NMR-data obtained from khellactone derivatives at a temperature of *ca.* 27°.

Compound	δ-values (ppm) for the *gem*-dimethyl groups	Coupling constants $J_{3',4'}$ (cps)	Solvent
trans-Khellactone (XII)	1.31 and 1.51	6.9 *	$CDCl_3$
trans-Diacetylkhellactone	1.38 and 1.46	4.4	$CDCl_3$
trans-Disenecioylkhellactone (XI)	1.33 and 1.43	3.5	CCl_4 or $CDCl_3$
trans-Methylkhellactone (IX)	1.40 and 1.48	3.0	$CDCl_3$
cis-Khellactone (VIII)	1.41 and 1.44	5.0 *	$CDCl_3$
cis-Disenecioylkhellactone (II)	1.40	5.0	CCl_4
Pteryxin (I)	1.42	5.0	CCl_4
cis-3'-Acetoxy-4'-senecioyloxy-3',4'-dihydroseselin (III)	1.39	5.0	CCl_4
cis-Methylkhellactone (X)	1.43	5.0	$CDCl_3$

* Hydroxylic hydrogen exchanged with deuterium.

almost unaltered, when passing from one conformer to the other. The value $J_{3',4'} = 5$ cps is somewhat higher than observed for 3,4-*cis*-disubstituted flavans. (2,3-*cis*-3,4-*cis*: $J_{3,4} = 3.9$—4.6 cps and 2,3-*trans*-3,4-*cis*: $J_{3,4} = 3.1$—3.5 cps [7]).

The other blue-fluorescent fraction obtained from the root was repeatedly chromatographed and afforded compound (IV) as a colourless glassy substance. All attempts to crystallize it were unsuccessful. Its UV-spectrum was very similar to that of simple 7-oxygenated coumarins. The NMR-spectrum was almost identical to that of jatamansin (XVI),[8] the angeloyl ester of lomatin (XVII),[9] the only remarkable difference being the presence of signals attributable to *para* protons in a benzene ring instead of those arising from the *ortho* protons in jatamansin. On saponification with methanolic sodium hydroxide compound (IV) afforded angelic acid, partly isomerized to tiglic acid, and a crystalline optically active alcohol (V) isomeric with lomatin (XVII). A comparison of the NMR-spectra revealed that, when going from compound (V) to its acetate, a signal due to a methine proton at δ 3.9 moved to a lower frequency by 1.2 ppm, which is indicative of the alcohol group present in compound (V) being secondary.[10] The evidence presented above suggested the structural possibilities (V) or (VI) for the optically active alcohol. The structure (VI), however, appeared to be less likely particularly because an ester of this benzylic type of alcohol presumably would form a methyl ether on treatment with methanolic sodium hydroxide, similar to what has been observed for khellactone esters.

The racemic alcohols (±)-3'-hydroxy-3',4'-dihydroxanthyletin and (±)-4'-hydroxy-3',4'-dihydroxanthyletin (VI) were prepared from natural xanthyletin (VII). A comparison with this synthetic material finally proved the structure (−)-3'-hydroxy-3',4'-dihydroxanthyletin (V) for the alcohol, obtained from (IV). This in turn established the structure (−)-3'-angeloyloxy-3',4'-dihy-

droxanthyletin for compound (IV).* A possible contamination of (IV) with the corresponding tigloyl ester, was ruled out by the absence in the NMR-spectrum of signals, corresponding to the vinyl proton in a tiglate.[11,12]

Another possibility of compound (IV) possessing the structure (XVIII) and being transformed into (V) by a cyclization reaction during the saponification is excluded by the fact, that in the NMR-spectrum of the acetate of (V) both parts of the ABX-system provided by the protons at the positions 3′ and 4′ are identical with respect to frequency and pattern to signals present in the spectrum of compound (IV). In the NMR-spectrum of ostholoxide (XIX), which shows structural features similar to those present in the structure (XVIII), the signals attributable to the protons at the positions 1′ and 2′ form an ABC-pattern in the region δ 2.5—3.5; furthermore, the *gem*-dimethyl group in this case gives rise to two separate signals (δ 1.30 and 1.50).

The preparation of (±)-3′-hydroxy-3′,4′-dihydroxanthyletin was performed by reduction of the corresponding ketone (XX), which was, in turn, obtained from (±)-*trans*-3′-hydroxy-4′-benzoyloxy-3′,4′-dihydroxanthyletin (XXI) by a treatment with boiling 10 N sulphuric acid. The benzoyl ester (XXI), obtained as the main product from an attempted epoxidation of xanthyletin (VII) with perbenzoic acid, presumably resulted from an attack of benzoic acid on a primarily formed epoxide. Styrene oxide is known to be attacked by organic acids exclusively at the carbon atom α to the phenyl group, thereby being transformed into α-monoesters. In some cases these esters have been shown to rearrange partly into the β-monoesters.[13] The reaction of organic acids with optically active styrene oxide is known to proceed with extensive racemization with a slight excess of inversion or retention dependent on the acid and solvent used.[14] From this it appeared that nothing could be anticipated concerning the position of the benzoyl group and the relative configuration in (XXI).

The assumption that (XXI) was the 4′-benzoate was confirmed by its extremely rapid alkaline methanolysis and by the rather low field position (δ 6.19) in its NMR-spectrum of the doublet arising from the proton at position 4′. The coupling constant $J_{3',4'} = ca.$ 6.5 cps and the two well separated signals from the *gem*-dimethyl group (δ 1.42 and 1.57), when compared to NMR-spectra of khellactone derivatives, established the relative configuration *trans* for compound (XXI).

The 2,4-dinitrophenylhydrazone of (XX) showed a light-absorption maximum at 346 mμ, indicating the parent ketone to be nonconjugated.[15,16] Nevertheless, the UV-spectrum obtained from (XX) itself differed from what is normally found for simple 7-oxygenated coumarins by an additional maximum

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* During the preparation of this manuscript the work on the constitution of decursin, a coumarin from *Angelica decursiva* Fr. *et* Sav., came to the authors' attention (K. Hata and K. Sano *Tetrahedron Letters* **1966** 1461).

Decursin, m.p. 111°, $[\alpha]_D^{15}$ + 172.9° ($CHCl_3$), is shown to be the senecioyl ester of optically active 3′-hydroxy-3′,4′-dihydroxanthyletin (m.p. 177°). The optical rotation values of this alcohol and its acetate (m.p. 139°) are not reported. The opposite signs, however, of the rotation values of decursin and of the angeloyl ester described in the present paper appear to indicate opposite configurations.

at 296 mμ. This is quite parallel to what has been described by Schroeder *et al.*[3] for 3′-keto-3′,4′-dihydroseselin.

(±)-4′-Hydroxy-3′,4′-dihydroxanthyletin (VI) was prepared by acid catalyzed hydration of xanthyletin. The acetate of (VI) was shown to cleave extremely rapidly on treatment with methanolic potassium hydroxide at room temperature.

EXPERIMENTAL

The plant material was collected in August on the coast of the Danish island Funen.

Isolation of the coumarin mixture. The dried and ground root (730 g) on extraction with ether and subsequent evaporation of the solvent afforded 81 g of oily extract, which after dilution with 90 % methanol was freed of lipids by extraction with petroleum ether.

Gross fractionation of the coumarin mixture. The defatted extract (31 g) was chromatographed on 550 g of silica gel (10 % of water) with benzene, benzene-chloroform mixtures, and chloroform as the eluents. A part of the material eluted was rechromatographed using the same conditions. These fractionations divided the material into three main fractions A, B, and C, mentioned in the order eluted.

Elution of the columns with methanol afforded further material (fraction D), which was not investigated.

Fraction A (0.15 g) was chromatographed on 20 g of silica gel (3 % of water). With benzene, and benzene to which ethyl acetate was added, until a concentration of 10 % was reached, the following crystallizable substances were eluted:

a. 4 mg of a yellow-fluorescent compound, m.p. 189—190° (methylene chloride-petroleum ether). The IR-data were in close agreement with those published by Perel'son [17] for bergapten (Ref. 18, m.p. 189—190°).

b. 40 mg of a bluish-fluorescent compound, m.p. 161—162.5° (methylene chloride-petroleum ether). The IR- and NMR-spectra were in close agreement with the data published by Perel'son [17] and Sheinker *et al.*,[19] respectively, for psoralen (Ref. 20, m.p. 161—163°; Ref. 21, m.p. 169—170°).

Fraction B (*ca.* 1 g) on trituration with ether-petroleum ether afforded 107 mg of a crystalline material and an oily residue. The crystals were purified by chromatography on 25 g of silica gel (3 % of water) with benzene, to which ethyl acetate in amounts gradually increasing to 1 % was added, as the eluent. 45 mg of a pure brown-fluorescent compound were obtained, m.p. 147.5—148° (methylene chloride-petroleum ether). The IR- and NMR-spectra were in agreement with those published by Perel'son [17] and Sheinker *et al.*[19] for xanthotoxin (Ref. 22, m.p. 145—146°).

The oily residue mentioned above was chromatographed several times on *ca.* 100-fold amounts of silica gel (3 % of water). As eluents were used benzene, and benzene to which ethyl acetate was added in amounts increasing to 10 %. The fractions eluted had to be analyzed by NMR-spectroscopy, as all thin-layer chromatographic procedures tried were inadequate. The following substances were obtained:

a. 270 mg of a blue-fluorescent glassy substance (IV) $[\alpha]_D^{25}$ −130°, $[\alpha]_{436}^{25}$ −300°, $[\alpha]_{364}^{25}$ −410° (*c* 0.5, $CHCl_3$); λ_{max} 328.5 mμ (4.19), 257 mμ (3.45), *ca.* 247 mμ (shoulder) (3.54), and 219 mμ (4.35); λ_{min} 263.5 mμ (3.13), 253 mμ (3.38), and 216 mμ (4.32).

The NMR-spectrum is shown in Fig. 1. The doublets at δ 6.06 and δ 7.51 (1 H each, J = 9.5 cps) are assigned to the protons at *a* and *b*. The peaks at δ 7.13 and δ 6.61 (1 H each) are assigned to the protons at the positions *c* and *d*,[19] the very broad pattern at δ 3 (2 H) and the pattern at δ 5 (1 H) to the protons at *e* and *f*, and the peak at δ 1.40 (6 H) to the protons in the *gem*-dimethyl grouping labelled *g*. The two methyl groups (*i* and *j*) in angelic acid give rise to the six-proton pattern at δ 2 and the vinyl proton labelled *h* to the very broad pattern at δ 6.

b. Ca. 250 mg of a glassy substance which from the NMR-spectrum appeared to consist mainly of (IV) in admixture with the corresponding senecioate and possibly also the isovalerate.

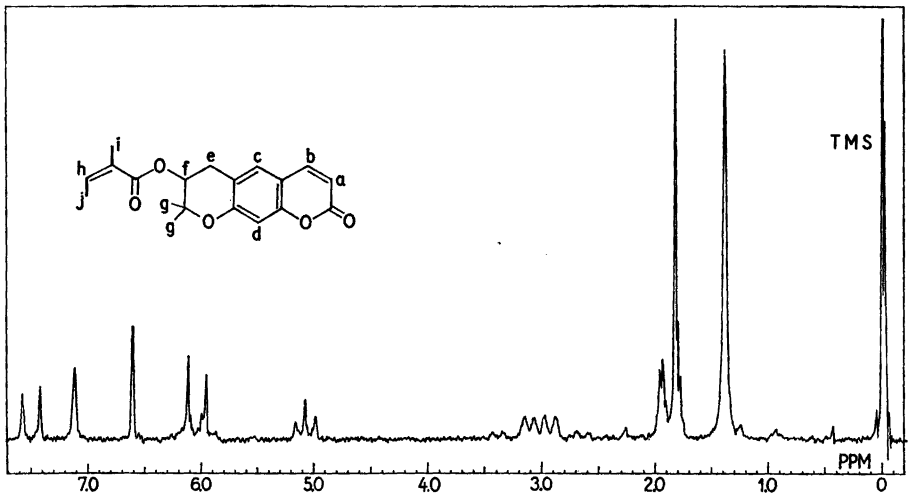

Fig. 1. NMR-spectrum (60 Mc/s) of (−)-3′-angeloyloxy-3′,4′-dihydroxanthyletin (IV) (tetrachloromethane). Internal standard, tetramethylsilane (TMS).

Fraction C (26 g) on thin-layer chromatographic analysis split up into five blue-fluorescent spots. Silica gel G (Merck) was used as the adsorbent. The eluent was tetrachloromethane-methylene chloride (1:2) to which ethyl acetate (6−8 %) was added. The travelling distance was *ca.* 10 cm. A deficient vapour saturation of the chamber, achieved for instance by omission of the paper lining, improved the separation. 2.4 g of the fraction was used in experiments described below. The remainder was chromatographed, and appropiate fractions rechromatographed several times on *ca.* 100-fold amounts of silica gel (3 % of water). As eluent was used tetrachloromethane-methylene chloride (1:2), to which ethyl acetate was added gradually until a concentration of 10 % was reached. In this way five fractions, which gave single spots with different mobility on thin-layer chromatograms, were isolated.

By NMR-spectroscopy the fractions were shown to be diesters of *cis*-khellactone. The fractions are mentioned in order of decreasing mobility on the plates.

a. Non-crystalline. The acid moieties were equal parts of angelic and isovaleric acids. The fraction is presumably a mixture of the two possible mixed esters.

b. A non-crystalline mixture, which on thin-layer chromatograms gave an elongated spot. The acid moieties were angelic, senecioic, isovaleric, and 2-methylbutyric acid.

c. A crystalline disenecioate (II), m.p. 108−108.5° (cyclohexane-petroleum ether), $[\alpha]_D^{25}$ + 15.8° (*c* 0.3, $CHCl_3$) (Found: C 67.64; H 6.05. Calc. for $C_{24}H_{26}O_7$: C 67.59; H 6.15).

d. Pteryxin (I), m.p. 86.5−88° (cyclohexane-petroleum ether) (Ref. 1, m.p. 81.5−82.5°), $[\alpha]_D^{25}$ + 9.8° (*c* 0.3 C_2H_5OH) (Ref. 1, $[\alpha]_D^{22}$ + 10° (C_2H_5OH)). The analytical data were concordant with the composition $C_{21}H_{22}O_7$. The IR-spectrum was identical with that of an authentic sample of pteryxin.

e. A crystalline mixed diester (III), the acid moieties of which were acetic and senecioic acids. M.p. 120.5−121° (ether-petroleum ether), $[\alpha]_D^{25}$ −11.9° (*c* 0.4, C_2H_5OH). (Found: C 65.49; H 5.81. Calc. for $C_{21}H_{22}O_7$: C 65.27; H 5.74).

The NMR-data (tetrachloromethane solution), from which the identity of the acids were concluded, are: A peak at δ 2.04 (3 H) corresponding to acetyl, two slightly broadened peaks at δ 1.88 and δ 2.19 (3 H each) corresponding to the two methyl groups, and a broad peak at δ 5.6 (1 H) corresponding to the olefinic proton in the senecioic acid moiety.

Methanolic saponification of compound (IV). 95 mg of compound (IV) were refluxed for 4 h with 5 ml of 1 N methanolic sodium hydroxide. The solution was diluted with water and acidified with 4 N sulphuric acid, finally adjusted to pH 8 with sodium car-

bonate solution, and extracted with methylene chloride. The extract was washed with water, a saturated solution of sodium chloride, dried, and evaporated. The crystalline residue was recrystallized from methylene chloride-ether to give 41 mg of the alcohol (V), m.p. 181–181.5°, $[\alpha]_D^{25}$ −13.0° (*c* 0.3, $CHCl_3$). (Found: C 68.53; H 5.60. Calc. for $C_{14}H_{14}O_4$: C 68.28; H 5.73).

Acetylation with acetic anhydride-pyridine gave a monoacetate, m.p. 140.5–141°, $[\alpha]_D^{25}$ −81° (*c* 0.3, $CHCl_3$).

The IR-spectrum of (V) (methylene chloride solution) showed no difference from that of (±)-3′-hydroxy-3′,4′-dihydroxanthyletin, synthetized as described below. Again, the NMR-spectra were identical, except for the positions of the signals arising from the hydroxylic protons (concentration dependent).

The aqueous phase was acidified and extracted with ether. The extract was washed with a saturated solution of sodium chloride and dried. Upon addition of 250 µl of dicyclohexylethylamine the solvent was evaporated. The *p*-phenylphenacyl ester was prepared according to Stodola [23] and isolated after chromatography on a silica gel column (15 g, 3 % of water). Elution of the column with benzene-petroleum ether (1:1) and later with benzene-petroleum ether (3:1) yielded the unreacted *p*-phenylphenacyl bromide. Elution with benzene afforded 17 mg of crystals, which on recrystallization from ethanol-water gave 9 mg of *p*-phenylphenacyl angelate, m.p. 88–89°. The IR-spectrum was identical with that of an authentic sample (m.p. 89–90°). Further elution with benzene gave 39 mg of crystals, which on recrystallization from ethanol-water afforded 26 mg of *p*-phenylphenacyl tiglate, m.p. 103.5–104.5°. The IR-spectrum was identical with that of an authentic sample (m.p. 104–105°).

Preparation of (+)-cis- *and* (−)-trans-*methylkhellactone (X, IX).* 1 g of fraction C was refluxed for 4 h with 1 N methanolic hydrochloric acid. The reaction mixture, liberated from acid, and evaporated to dryness was chromatographed on 80 g of silica gel (3 % of water) with benzene-ethyl acetate mixtures as the eluents. Obtained were:

a. 110 mg of (+)-*cis*-methylkhellactone (X), m.p. 125.5–126.5° (ether-petroleum ether), $[\alpha]_D^{25}$ + 80° (*c* 0.3, $CHCl_3$). (Ref. 3, m.p. 127–127.5°, $[\alpha]_D^{23}$ + 78°).

b. 310 mg of (−)-*trans*-methylkhellactone (IX), m.p. 162–162.5° (methylene chloride-ether), $[\alpha]_D^{25}$ −30° (*c* 0.4, $CHCl_3$). (Ref. 3, m.p. 161.5–162.5°, $[\alpha]_D^{26}$ −30°).

The analytical data and the NMR-spectra were consistent with the known constitutions.

*Preparation of (+)-*cis- *and (−)-*trans-*khellactone (VIII, XII).* A crude khellactone mixture was obtained from 1.4 g of fraction C by saponification in dioxane-water as described by Schroeder *et al.*[3] for the preparation of khellactones from "visnagan". The reaction product was chromatographed on silica gel (3 % of water) with benzene-methanol mixtures as the eluents. Obtained were:

a. 70 mg (+)-*cis*-khellactone (VIII), m.p. 174–175° (tetrachloromethane-methylene chloride), $[\alpha]_D^{25}$ + 75° (*c* 0.5, $CHCl_3$) (Ref. 3, m.p. 174–175°, $[\alpha]_D^{20}$ + 81°).

b. 180 mg (−)-*trans*-khellactone (XII), m.p. 187.5–188.5°; $[\alpha]_D^{25}$ −18° (*c* 0.4, $CHCl_3$) (Ref. 3, m.p. 185–186°, $[\alpha]_D^{21}$ −18°). The NMR-spectra were consistent with the known constitutions.

*Acid methanolysis of (+)-*cis-*disenecioylkhellactone (II).* 119 mg of (II) were refluxed with 2 N methanolic hydrochloric acid (10 ml). The disappearance of starting material and intermediates was followed by thin-layer chromatography. The reaction required 45 h for completion. The reaction mixture was distilled at reduced pressure at 25–80°C. The residue was dissolved in methylene chloride, washed with sodium hydrogen carbonate solution (used below), water, and saturated sodium chloride solution, dried, and evaporated to dryness. The mixture obtained was separated on silica gel (12 g, 5 % of water). Elution with benzene, to which ethyl acetate was gradually added, until a concentration of 20 % was reached, afforded:

a. 2 mg of a substance, which after recrystallization from ether-petroleum ether melted at 124–125°. The IR-spectrum was identical with that of (+)-*cis*-methylkhellactone (X) (m.p. 127–127.5°).

b. 17 mg of (−)-*trans*-methylkhellactone (IX), m.p. 161.5–162° (methylene chloride-ether) $[\alpha]_D^{25}$ −28° (*c* 0.45, $CHCl_3$).

2 N Sodium hydroxide was added to the distillate, obtained as described above, until a normality of about 0.5 was reached. After standing for 2 days the solution was acidified with sulphuric acid and adjusted to *ca.* pH 8 with sodium carbonate solution.

The sodium hydrogen carbonate solution mentioned above was added and the solution evaporated to dryness at reduced pressure. The residue was dissolved in 10 ml of water, acidified with 4 N sulphuric acid, and extracted with ether. The ether phase was washed with saturated sodium chloride solution, and dried. After addition of dicyclohexylethylamine the solvent was distilled off. The *p*-phenylphenacyl ester was prepared according to Stodola [23] and liberated from excess of reagent by chromatography on a silica gel column as described above.

45 mg of *p*-phenylphenacyl senecioate were obtained, m.p. 143.5—144.5° (ethanol) (Ref. 2, m.p. 144.5—146°). The IR-spectrum was identical with that of an authentic sample.

Preparation of (+)-trans-disenecioylkhellactone (XI). A mixture of (—)-*trans*-khellactone (77 mg), sodium senecioate (144 mg) and senecioic anhydride (1.0 ml) was maintained at 125° in a closed tube for 3 h. To the cooled reaction mixture ether and water were added. The ether phase was washed with sodium hydrogen carbonate solution, saturated sodium chloride solution, and dried. Upon evaporation of the solvent the mixture was chromatographed on 20 g of silica gel (5 % of water). The excess of senecioic anhydride was eluted with benzene-petroleum ether (3:1) and benzene; benzene, to which 5 % and later 10 % of ethyl acetate was added, eluted compound (XI).

The *cis*-disenecioate (II) and its *trans*-epimer (XI) were easily separable in the thin-layer chromatographic system described above for khellactone esters, the latter showing the greatest mobility. The *cis*-disenecioate (II) (or other side products) were not detectable in the reaction mixture or in appropiate concentrated column effluents. (XI) crystallized from cyclohexane with 0.5 mole of the solvent, this ratio being reproducible, and easily detectable from the NMR-spectra.

The yield was 93 mg; m.p. *ca.* 85 to *ca.* 95° (effervescence at *ca.* 100°), $[\alpha]_D^{25}$ + 125° (*c* 0.4, $CHCl_3$) (calculated for $C_{24}H_{26}O_7$). (Found: C 69.14; H 6.73. Calc. for $C_{24}H_{26}O_7$, 0.5 C_6H_{12}: C 69.21; H 6.88). The NMR-spectrum was consistent with the established structure.

Preparation of (II) from (+)-cis-khellactone. A mixture of (+)-*cis*-khellactone (30 mg), sodium senecioate (65 mg), and senecioic anhydride (0.50 ml) was heated to 120° in a closed tube for 4 h. The reaction mixture was treated and fractionated as described above for the *trans*-epimer. The reaction mixture contained a small amount of a blue-fluorescent side product, which was separable from (II) on thin-layer chromatograms. The *trans*-epimer was not present. The yield of (II) was 20 mg; m.p. 108.5—109° (cyclohexane-petroleum ether), $[\alpha]_D^{25}$ + 17.3° (*c* 0.2, $CHCl_3$). The identity with natural (II) was evident from the IR-spectrum.

Hydrogenation of compound (III). 145 mg of (III) in 96 % ethanol (35 ml) were hydrogenated at 30° with 70 mg of platinum oxide (Fluka) as a catalyst. After 22 min an amount of hydrogen corresponding to 1 mole had been absorbed. The hydrogenation was interrupted, and the catalyst and solvent removed.

The product was chromatographed on 20 g of silica gel (3 % of water). Elution with tetrachloromethane-methylene chloride (1:2) to which ethyl acetate was gradually added until a concentration of 5 % was reached, afforded suksdorfin (XV) (90 mg), m.p. 140—140.5° (Ref. 1, m.p. 140.5—141°), $[\alpha]_D^{25}$ —6.6°, $[\alpha]_{364}^{25}$ + 170° (*c* 0.3, $CHCl_3$). An authentic sample showed: $[\alpha]_D^{25}$ —5.8°, $[\alpha]_{364}^{25}$ + 175° (*c* 0.3, $CHCl_3$). The IR-spectrum was identical with that of the authentic sample.

Xanthyletin (VII) was isolated from commercial east indian satinwood *(Chloroxylon swietenia)*, which has formerly been shown [24] to be a good source of this compound.

The sample melted at 129.5—130° (methanol) (Ref. 24, 126—127°). λ_{max} 346.5 mμ (4.14), 302 mμ (3.8), 265.5 mμ (4.34), 225 mμ (4.42). King *et al.*[24] reported λ_{max} 348 mμ (4.15) and 266 mμ (4.34). The analytical data were concordant with the elemental composition $C_{14}H_{12}O_3$ and the NMR-spectrum consistent with the known structure.

Epoxydation of xanthyletin (VII). 5.5 ml of a 0.53 M solution of perbenzoic acid in ether were added to 0.6 g of xanthyletin (VII) dissolved in 5 ml of chloroform. After standing for 3 days at room temperature, the solution was diluted with ether, washed with sodium hydrogen carbonate solution and a saturated solution of sodium chloride, and finally dried. After evaporation of the solvent the residue was chromatographed on 35 g of silica gel (10 % of water). Benzene, to which increasing amounts of ethyl acetate were added, and finally pure ethyl acetate, were the eluents employed.

In addition to oily material the following crystalline substances were obtained:

a. (±)-*trans*-3′-hydroxy-4′-benzoyloxy-3′,4′-dihydroxanthyletin (XXI) (169 mg), m.p. 183–183.5°. (Found: C 68.69; H 4.87. Calc. for $C_{21}H_{18}O_6$: C 68.84; H 5.09).

b. 70 mg of a substance, m.p. 181–191°, which is presumably a mixture of (±)-*cis*- and (±)-*trans*-3′,4′-dihydroxy-3′,4′-dihydroxanthyletin. (Found: C 64.09; H 5.36. Calc. for $C_{14}H_{14}O_5$: C 64.11; H 5.38).

A solution of (XXI) in 1 N methanolic potassium hydroxide was acidified after standing for 5 min at room temperature. Thin-layer chromatographic analysis of the reaction mixture on silica gel G (Merck), with benzene-methanol (9:1) as the eluent, revealed the complete disappearance of starting material. Instead a double spot with lower mobility, presumably corresponding to the epimeric methyl ethers, and a comparably much smaller spot, corresponding to the glycol mixture mentioned above, appeared.

3′-Keto-3′,4′-dihydroxanthyletin (XX). A solution of 115 mg (XXI) in ethanol (20 ml) and 10 N sulphuric acid (80 ml) was refluxed for 4.5 h. The cooled reaction mixture was extracted with benzene, the benzene extract washed with sodium hydrogen carbonate solution and a saturated sodium chloride solution, and finally dried with anhydrous sodium sulphate. After evaporation of the solvent the residue was chromatographed on 12 g of silica gel (10 % of water). The eluent was benzene, to which ethyl acetate was added gradually, until a concentration of 10 % was reached. 3′-Keto-3′,4′-dihydroxanthyletin (XX) (42 mg), m.p. 164–165.5° (tetrachloromethane-methylene chloride) was obtained. (Found: C 68.76; H 4.93. Calc. for $C_{14}H_{12}O_4$: C 68.89; H 4.95). λ_{max} 327 mμ (4.19), 296 mμ (4.02). λ_{min} 303 mμ (3.99), 263 mμ (2.56) (chloroform). In the NMR-spectrum, which was consistent with the established structure, the signal arising from the methylene group appeared at δ 3.70 ($CDCl_3$). The 2,4-dinitrophenylhydrazone, prepared according to Neuberg *et al.*,[25] melted at 253–254° (decomp.). A crude product, obtained by an attempted epoxidation of 560 mg of xanthyletin (VII), on treatment with 10 N sulphuric acid and subsequent fractionation as described above, afforded 372 mg of (XX), m.p. 162.5–165.5°.

(±)-3′-Hydroxy-3′,4′-dihydroxanthyletin. 299 mg of (XX), m.p. 162.5–165.5°, was dissolved in 75 ml of isopropanol, which contained 0.4 % of water. The solution was cooled to 0° and, after the addition of 300 mg of sodium borohydride, maintained at this temperature for 15 min. The temperature was then quickly raised to 25°, and after further 15 min the mixture was acidified with 1 N hydrochloric acid. During the process the flask was steadily agitated. After dilution with water, the solution was extracted two times with benzene. The benzene extracts were washed with sodium hydrogen carbonate solution, followed by a saturated sodium chloride solution. After filtration through anhydrous sodium sulphate, the benzene solution was evaporated to dryness. The crystalline residue was recrystallized from ether-methylene chloride, to give (±)-3′-hydroxy-3′,4′-dihydroxanthyletin (236 mg); m.p. 167.5–168.5°. (Found: C 68.42; H 5.83. Calc. for $C_{14}H_{14}O_4$: C 68.28; H 5.73).

(±)-4′-Hydroxy-3′,4′-dihydroxanthyletin (VI). To 400 mg of xanthyletin (VII), dissolved in acetic acid (10 ml), concentrated hydrochloric acid (10 ml) was added. After standing for 6 days at room temperature the solution was diluted with water and extracted twice with methylene chloride. The extracts were washed with water, sodium hydrogen carbonate solution and saturated sodium chloride solution, and finally dried. After evaporation of the solvent the residue was chromatographed on 25 g of silica gel (10 % of water). As the eluent was used benzene, to which increasing amounts of ethyl acetate (0–30 %) were gradually added. The following substances were obtained:

a. 181 mg of unreacted xanthyletin.

b. 57 mg of a blue-fluorescent substance, m.p. >300°, which was not further investigated.

c. 52 mg of compound (VI), m.p. 167–168°. (Found: C 68.17; H 5.64. Calc. for $C_{14}H_{14}O_4$: C 68.28; H 5.73).

The acetate of (VI) was prepared by the action of acetic anhydride-pyridine at room temperature overnight; m.p. 142.5–143.5°.

A solution of this acetate in 1 N methanolic potassium hydroxide was acidified after standing for 5 min at room temperature. Thin-layer chromatographic analysis of the reaction mixture revealed the complete disappearance of the acetate. Instead a blue-fluorescent spot with a slightly lower mobility, presumably corresponding to the methyl ether, a spot corresponding to xanthyletin, and a very small spot corresponding to the alcohol (VI), appeared. Silica gel G (Merck) was used as the adsorbent, tetrachloro-

methane-methylene chloride (1:2) to which 6 % of ethyl acetate had been added, as the eluent.

Ostholoxide (XIX), m.p. 105.5—106.5°, was prepared from osthol according to Späth *et al.*[26]

The silica gel (Merck, 0.05 mm — 0.20 mm) used in column chromatographic separations was activated at 120° overnight prior to use, and then mixed with the amount of water stated for each experiment.

Melting points, UV-, IR-, and NMR-spectra were determined as described in a previous paper.[27] Microanalyses were performed by Dr. A. Bernhardt, Mülheim.

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REFERENCES

1. Wilette, R. E. and Soine, T. O. *J. Pharm. Sci.* **51** (1962) 149.
2. Smith, E., Hosansky, N., Bywater, W. G. and van Tamelen, E. E. *J. Am. Chem. Soc.* **79** (1957) 3534.
3. Schroeder, H. D., Bencze, W., Halpern, O. and Schmid, H. *Chem. Ber.* **92** (1959) 2338.
4. Whalley, W. B. In Geissman, T. A. *The Chemistry of Flavonoid Compounds*, Pergamon, London 1962, p. 440.
5. Clark-Lewis, J. W., Jackman, L. M. and Spotswood, T. M. *Australian J. Chem.* **17** (1964) 632.
6. Corey, E. J., Philbin, E. M. and Wheeler, T. S. *Tetrahedron Letters* **1961** 429.
7. Jackman, L. M. In Zechmeister, L. *Fortschr. Chem. org. Naturstoffe* **23** (1965) 315.
8. Shanbhag, S. N., Mesta, C. K., Maheshwari, M. L., Paknikar, S. K. and Bhattacharyya, S. C. *Tetrahedron* **20** (1964) 2605.
9. Soine, T. O. and Jawad, F. H. *J. Pharm. Sci.* **53** (1964) 990.
10. Jackman, L. M. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon, New York 1959, p. 55.
11. Fraser, R. R. *Can. J. Chem.* **38** (1960) 549.
12. Wilette, R. E. and Soine, T. O. *J. Pharm. Sci.* **53** (1964) 275.
13. Cohen, T., Dughi, M., Notaro, V. A. and Pinkus, G. *J. Org. Chem.* **27** (1962) 814.
14. Berti, G., Bottari, F., Ferrarini, P. L. and Macchia, B. *J. Org. Chem.* **30** (1965) 4091.
15. Braude, E. A. and Jones, E. R. H. *J. Chem. Soc.* **1945** 498.
16. Roberts, J. D. and Green, C. *J. Am. Chem. Soc.* **68** (1946) 214.
17. Perel'son, M. E. *Zh. Obshch. Khim.* **33** (1963) 952.
18. Späth, E., Wessely, F. and Kubiczek, G. *Ber.* **70** (1937) 478.
19. Sheinker, Y. N., Pek, G. Y. and Perel'son, M. E. *Dokl. Akad. Nauk SSSR* **158** (1964) 1382.
20. Jois, H. S., Manjunath, B. L. and Rao, S. V. *J. Indian Chem. Soc.* **10** (1933) 41.
21. Späth, E., Manjunath, B. L., Pailer, M. and Jois, H. S. *Ber.* **69** (1936) 1087.
22. Thoms, H. *Ber.* **44** (1911) 3325.
23. Stodola, F. H. *Microchem. J.* **7** (1963) 389.
24. King, F. E., Housley, J. R. and King, T. J. *J. Chem. Soc.* **1954** 1392.
25. Neuberg, C., Grauer, A. and Pisha, B. V. *Anal. Chim. Acta* **7** (1952) 238.
26. Späth, E., Eiter, K. and Meinhard, Th. *Ber.* **75** (1942) 1623.
27. Nielsen, B. E. and Lemmich, J. *Acta Chem. Scand.* **18** (1964) 1379.

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