ANOMALIN, A NEW NATURAL PYRANOCOUMARIN

E. B. Zorin, G. K. Nikonov, and G. Yu. Pek

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 1, pp. 3-7, 1967

In a study of the roots of Angelica anomala Avé-Lall. (eumenol angelica), family Umbelliferae, by absorption chromatography on alumina we have isolated a neutral substance $C_{24}H_{26}O_7$ with mp 177.5-178.5°C, whose properties are those of a lactone of the coumarin group [1, 2].

The UV spectrum of the substance (Fig. 1) has absorption maxima at 225, 255, and 320 mμ (log ε > 4.34, 3.58, 4.26), which confirms the presence of a coumarin structure [3, 4]. The spectrum, in particular the low absorption at 255 mμ, resembles the spectrum of known dihydropyrano- and dihydrofuranocoumarins [5-9].

The lactone does not contain methoxy or hydroxy groups and does not react with carbonyl reagents. Its IR spectrum (Fig. 2) exhibited absorption bands of the carbonyl group of an α, β-lactone conjugated with an aromatic nucleus (1735 cm^{-1}) and of an aromatic nucleus (1610, 1580, 1470 cm^{-1}). The empirical formula and the physicochemical constants give grounds for regarding this as a new coumarin, and we have called it anomalin.

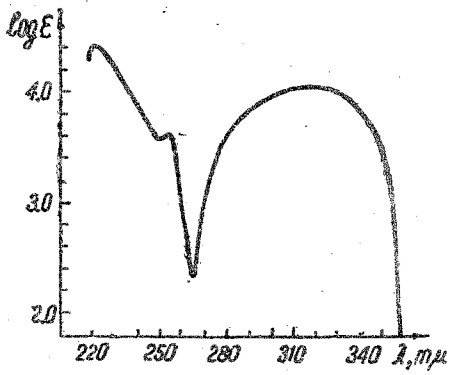


Fig. 1. UV spectrum of anomalin (alcoholic solution).

On catalytic hydrogenation over platinum oxide (Adams), at normal pressure and room temperature, one mole of anomalin added two moles of hydrogen, forming the tetrahydro derivative $C_{24}H_{30}O_7$, which shows the presence of two double bonds not conjugated with an aromatic nucleus. The IR spectrum of the compound obtained has absorption bands at 1740, 1610, 1577, and 1469 cm^{-1}, showing that the coumarin skeleton undergoes no change on hydrogenation and that the two double bonds in the initial substance are present in a side chain. The presence of double bonds in the initial substance was also confirmed by the preparation of a tetrabromo derivative $C_{24}H_{26}Br_4$.

The broad carbonyl band in the IR spectrum of anomalin and the change in the R_f value after treatment with alkali permit the assumption that it is an ester. The hydrolytic decomposition of anomalin with a solution of caustic soda in methanol gave a hydroxy lactone with the composition $C_{15}H_{16}O_5$ the IR spectrum of which had absorption bands of a hydroxy group (3450 cm^{-1}), a lactone carbonyl group (1707 cm^{-1}) and an aromatic nucleus (1612, 1575, 1472 cm^{-1}). The hydroxy lactone contained one hydroxy and one methoxy group and by its composition, a mixed melting point test, and its IR spectrum it was identified as 2', 2'-dimethyl-3'-hydroxy-4'-methoxy-5'-, 6': 8, 7-coumarin (methylkhellactone), a known product of the hydrolytic decomposition of dihydrosamidin, visnadin, steryxin, etc. [9-11].

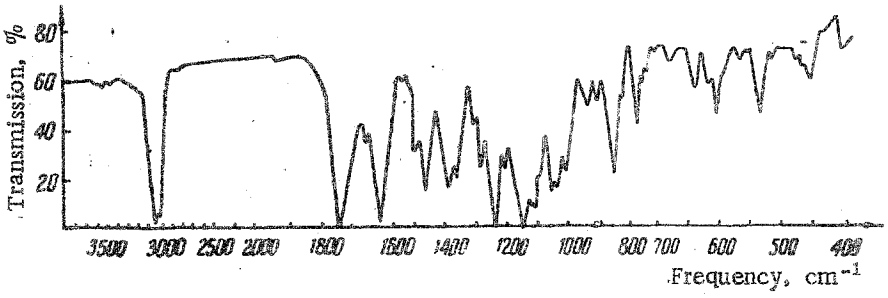
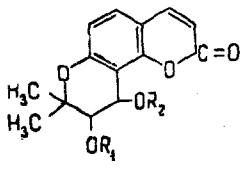


Fig. 2 IR spectrum of anomalin (mull in paraffin oil).

As Späth, Soine, and Smith have shown [9-11], the dihydroxycoumarin khellactone is present in plants in the form of esters with acetic, isovaleric, and angelic acids. The esters of khellactone are characterized by the different stabilities of the ester groups at C_3' and O_4'. A bond at C_4' possesses enhanced reactivity and, in those cases where hydrolysis is carried out in methyl or ethyl alcohol, etherification of the hydroxy group appearing at C_4' takes place; the final product is not khellactone (I) (2', 2'-dimethyl-3', 4'-dihydroxy-3', 4'-dihydropyrano-5', 6': 8, 7-coumarin), but methyl-(II) or

ethylkhellactone (III) 2', 2'-dimethyl-2'-hydroxy-4'-methoxy - or -4'-ethoxy-3', 4'-dihydropyrano-5', 6':8, 7-coumarin).

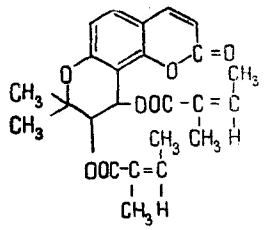


I R_1, R_2 – H
II R_1 – H, R_2 – CH_3
III R_1 – H, R_2 – C_2H_5

Thus, anomalin may be considered as a monester of methylkhellactone and an unsaturated acid or as a diester of khellactone. The first hypothesis is excluded since anomalin does not contain a methoxy group and, consequently, it may be a diester of khellactone.

For a definitive proof of the structure of anomalin, we studied the composition of the acids present in the 3' and 4' positions. The acid fraction obtained by the hydrolytic decomposition of anomalin was studied by chromatography on paper in the following systems: 1) butan-1-ol saturated with water; 2) butan-1-ol saturated with a 1.5 N solution of ammonia; and 3) amyl alcohol–acetic acid–water (4: 1: 5) [12]. The chromatograms were revealed with a 0.2% alcoholic solution of bromophenol blue. On spraying, all chromatograms showed a single spot with the R_f values 0.54 in system 1, 0.53 in system 2, and 0.50 in system 3, which correspond to angelic or tiglic acid.

The acid isolated, with the composition $C_5H_8O_2$, was identified by its IR spectrum as trans-1, 2-dimethylacrylic (angelic) acid. The latter is the labile form of 1, 2-dimethylacrylic acid and when its aqueous solutions are subjected to prolonged heating it isomerizes irreversibly into tiglic acid. The isolation of spectrally pure (IR spectrum) angelic acid showed that this acid esterified both hydroxy groups of the khellactone. Consequently, anomalin is 2', 2'-dimethyl-3', 4'-diangeloyl-3', 4'-dihydropyrano-5', 6':8, 7-coumarin and has the following structure:



To confirm the structure found, the NMR spectrum of anomalin was recorded. In the 6–8 ppm* region of the NMR spectrum there are eight multiplets which were determined on the basis of previous work on the NMR spectroscopy of the coumarins [13–15]. From these peaks, the quadruplets j and n (δ = 6.01 and 7.04 ppm, J = 9.1 Hz) are due to the C_3 and C_4 protons of the pyran ring, and m and *l* (δ = 7.20 and 6.60 ppm, J = 9.0 Hz) to the C_5 and C_6 protons of the benzene ring. Consequently, anomalin is a 7, 8-substituted coumarin. The two-proton quadruplets g and k (δ = 5.25 and 6.49 ppm, J = 5.0 Hz) correspond to the C_3', and C_4' protons of the pyran ring.

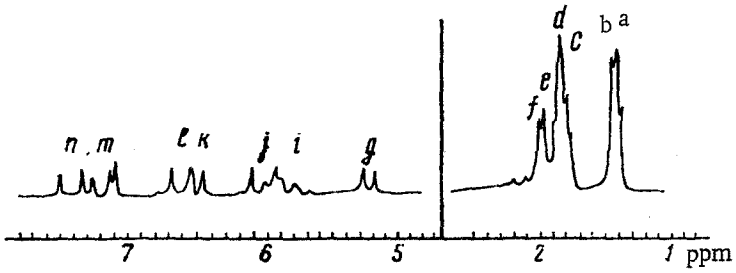


Fig. 3. NMR spectrum of anomalin (in chloroform solution).

Fraser, Seshadri, and Khata [16–18] have established that by using the magnitude of the chemical shift it is possible to distinguish the angelates of coumarins from their tiglates. The vinyl proton of angelic acid resonates in the region δ = 5.90–5.98 ppm, while the corresponding proton of tiglic acid does so in the region δ = 6.50 ppm. The multiplet i (δ = 5.90 ppm) showed that anomalin is an angelate.

*The chemical shifts were calculated in parts per million with respect to tetramethylsilane as internal standard taken as zero.

In the 1–2 ppm region there are two three-proton peaks a and b (δ = 1.41 and 1.44 ppm), which are characteristic for ethyl groups in a pyran ring, and the multiplets c, d, e, f (δ = 1.70–2.20 ppm) corresponding in intensity, position, and splitting to the methyl groups of two angelic acid residues.

These results confirm the above conclusion on the structure of anomalin.

Experimental

Isolation of anomalin. Five kilograms of the dried and comminuted roots were extracted with cold methanol (30, 24, and 24 *l*). The extract was concentrated in vacuum to 2.5 *l* and treated with 5 *l* of water. The mixture was extracted with four 1-*l* portions of ether, and the ethereal extracts were combined, evaporated to 700 ml, and washed with a 0.5% aqueous solution of caustic potash until the phenols had been removed completely, after which the solvent was distilled off. The residue (82 g) was mixed with an equal amount of alumina and transferred to a column of acid alumina (60 × × 6.5 cm, Brockmann activity grade II). The column was washed with 5 *l* of benzene–methanol (99:1). Concentration of the eluate gave a colorless crystalline substance. Yield 11 g (0.22%). The substance was readily soluble in chloroform and ether, sparingly in alcohol, and insoluble in water, mp 177.5–178.5°C (from methanol), $[\alpha]_D^{22}$ −50° (c 1.0; chloroform), R_f 0.95 [chromatographed on paper impregnated with a 10% solution of formamide in methanol in the n-hexane–benzene–methanol (5:4:1) system, with diazotized sulphanilamide as the revealing agent [12]]. IR spectrum: 1735, 1610, 1580, 1470, 1386, 1364, 1240, 1110, 855 cm^{-1}.

Found, %: C 67.70; 67.57; H 6.52; 6.17; C–CH_3 9.18; mol. wt. 423, 424 (Beckmann). Calculated for $C_{24}H_{26}O_7$, %: C 67.59; H 6.14; C–CH_3 3.52; mol. wt. 426.47.

Tetrahydro derivative. A solution of 0.465 g of anomalin in 50 ml of methanol was hydrogenated in the presence of 0.0811 g of platinum oxide. The absorption of hydrogen ceased after 40 min, when 48 ml of hydrogen had been consumed. The alcoholic solution was filtered to remove the catalyst and evaporated to dryness. Colorless acricular crystals with mp 135–137°C (from methanol) were formed. IR spectrum: 1740, 1610, 1574, 1469, 1390, 1358, 1240, 1100, 850 cm^{-1}.

Found, %: C 67.23; 67.41; H 6.72; 6.74. Calculated for $C_{24}H_{30}O_7$, %: C 67.27; H 7.05.

Tetrabromo derivative. A 1% solution of bromine in chloroform was added to 0.25 g of anomalin in 10 ml of chloroform until a faint pink coloration was produced. After the solvent had been distilled off, Colorless acicular crystals with mp 106–108°C (from 70% methanol) were obtained.

Found, %: C 38.66; 38.76; H 3.63; 3.56; Br 42.71; 42.68. Calculated for $C_{24}H_{26}O_7Br_4$, %: C 38.63; H 3.51; Br 42.84.

Methylkhellactone. A solution of 1 g of anomalin in 30 ml of a 5% methanolic solution of caustic potash was heated in a water bath for 3.5 hr and was then diluted with 60 ml of water and acidified with sulfuric acid. The mixture was treated with three 25-ml portions of ether. The solvent was distilled off to give colorless crystals with mp 162–163° C (from methanol), $[\alpha]_D^{22}$ + 16° (c 1.0; chloroform), R_f 0.15. IR spectrum: 3450, 1707, 1612, 1575, 1472, 1390, 1373, 1240, 1100, 850 cm^{-1}.

Found, %: C 65.60; 65.59; H 5.96; 5.99; OCH_3 11.71; 11.88; H_{labile} 0.43, 0.41. Calculated for $C_{15}H_{16}O_5$, %: C 65.21; H 5.83; OCH_3 11.23; H_{labile} 0.36.

Angelic acid. One gram of anomalin was saponified by the method given above. The alkaline solution was diluted with water and the methyl alcohol was distilled off in vacuum. The liquid was acidified with sulfuric acid and the methylkhellactone which separated out was filtered off. The filtrate was treated with three 25-ml portions of ether. The solvent was distilled off. A syrupy liquid with a specific odor remained, which slowly crystallized. IR spectrum: 3526, 1695, 1650, 1465, 1424, 1385, 1355, 1280, 1165, 1090 cm^{-1}.

Found, %: C 60.29; 60.06; H 8.77; 8.71; H_{labile} 0.95, 1.05. Calculated for $C_5H_8O_2$, %: C59.99; H 8.05; H_{labile} 1.01.

The NMR spectrum was taken on a JEOL NMR spectrometer (60 MHz). The UV and IR spectra were taken by M. E. Perel'son on a SF-4 spectrophotometer and a UR-10 infrared spectrophotometer.

Summary

A new coumarin $C_{24}H_{26}O_7$, which we have called anomalin has been isolated from the roots of Angelica anomala Avé-Lall. On the basis of its chemical properties, the results of a study of its saponification products and UV, IR, and NMR spectroscopy it has been established that anomalin is 2', 2'-dimethyl-3', 4'-diangeloyl-3', 4'-dihydropyrano-5', 6': 8, 7-coumarin.

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16 April 1966

Sechenov First Moscow Medical Institute;
Institute of the Chemistry of
Natural Compounds AS USSR