UDC 547.814

COUMARINS OF THE LIBANOTIS BUCHTORMENSIS

N. E. Ermatov, A. I. Ban'kovskii, M. E. Perel'son, G. P. Syrova, and Yu. N. Sheinker

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 3, pp. 145-149, 1968

Libanotis buchtormensis (Fisch.) D. C. is a perennial herbaceous plant of the family Umbelliferae which grows in the mountain regions of Central Asia and western and eastern Siberia [1].

This species has not been subjected to a detailed chemical study; the only information in the literature is that its fruit contains 1% of total coumarins [2] consisting, according to paper chromatography, of five substances [3].

We have studied the roots of Libanotis buchtormensis collected by D. A. Pakalnyi in the region of the western Altai (district of Lake Kolyvanskii) in 1964 and 1965.

The total coumarins in the roots and herbaceous part of the plant amounted to 2.02 and 1.05%, respectively (weight method). Paper chromatography showed that they consisted of at least six substances. By extracting the roots with methanol and subsequent chromatography on alumina, we isolated isoimperatorin and bergapten. By chromatographic separation on alumina of an ethereal extract of the roots, we obtained xanthogallol (I) [4].

By preparative thin-layer chromatography, from an ethereal extract of the roots of Libanotis buchtormensis we isolated a chromatographically-homogeneous substance (II) with R_f 0.35 (TLC, system 1) with the composition $C_{19}H_{20}O_5$; $[\alpha]_D^{18}$ +50°, readily soluble in chloroform, ethanol, and ethyl acetate and sparingly soluble in petroleum ether. Attempts to crystallize it from various solvents proved unsuccessful.

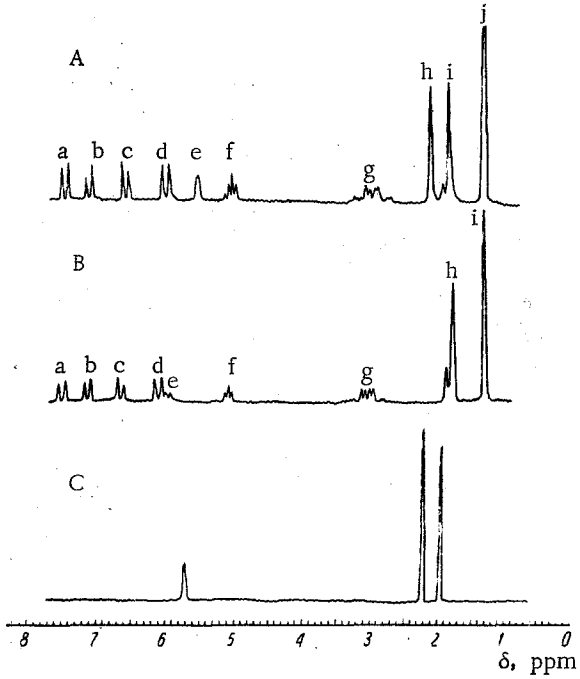


Fig. 1. NMR spectra of substance (II) (A) (in CCl_4), xanthogallin (B), and β, β-dimethylacrylic acid (C) (in $CDCl_3$).

The compound exhibited the characteristic properties of coumarins: it readily dissolved in ethanolic alkali, forming a lemon-yellow solution; it fluoresced violet on irradiation with ultraviolet light, and gave a bright pink color on treatment with Pauly's reagent. The substance contained no hydroxy or methoxy groups. Its IR spectra was typical for coumarins, and the high intensity of the carbonyl band did not exclude the presence of an ester grouping in the molecule. In actual fact, when substance II was saponified in ethanolic alkali, xanthogallol (I) was obtained.

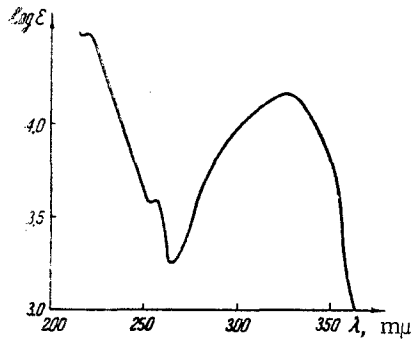


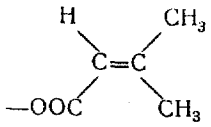
Fig. 2. UV spectrum of buchtormin in 96% ethanol.

Thus, the substance (II) that we isolated is isometric with xanthogallin (III) [5] and gives the same hydroxy lactone on saponification. It differs from xanthogallin by the structure of the acid residue.

From a comparison of the IR spectra of xanthogallin and substance (II) in chloroform it can be seen that they differ very slightly.

A study of the NMR spectrum of substance (II) showed that it consisted of a mixture of two isomeric compounds—xanthogallin and a new coumarin which we have called buchtormin, this being an ester of xanthogallol and β, β-dimethylacrylic acid. In the region of aromatic protons in the spectrum of substance (II) (Fig. 1, A and B) the same pattern is observed as in the spectrum of xanthogallin [4]; the doublets a, b, c, and d are due, respectively, to the protons in positions 4, 5, 6, and 3 of the coumarin nucleus. The triplet f and the multiplet g from the methyl and methylene protons of the dihydropyran ring and also the signals from the two methyl groups in the spectra of substance (II) and xanthogallin have similar chemical shifts and spin-spin interaction constants. All this is in harmony with the formation of xanthogallol by the saponification of substance (II).

The peaks h (δ = 2.10) and i (δ = 1.83) and the signal e (δ = 5.56) relate to the β, β-dimethylacrylic acid residue



Actually, the positions of the two somewhat broadened peaks h and i correspond to methyl groups on a double bond and signal e to a single proton on a double bond experiencing weak allyl splitting through interaction with the methyl groups. This assignment is also confirmed by a comparison of the spectra of substance (II) and β, β-dimethylacrylic acid (Fig. 1, C). The features of the spectrum of β, β-dimethylacrylic acid given agree with the results published in the literature [6].

Thus, substance (II) consists mainly of buchtormin—the ester of xanthogallol and β, β-dimethylacrylic acid.

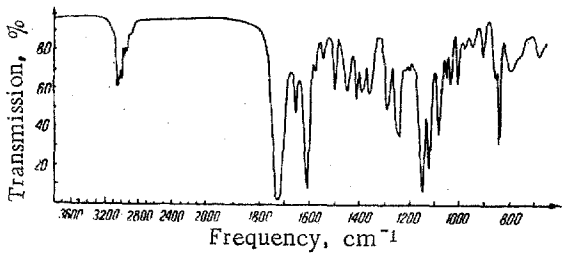


Fig. 3. IR spectrum of buchtormin in $CHCl_3$ (d = 0.1 mm).

In addition to buchtormin, substance (II) contained a small amount of xanthogallin, as was shown by the splitting and slight broadening of peak i due to the methyl groups of angelic acid (see Fig. 1, A and B) and also by the appearance of a diffuse signal at peak d in the position of the multiplet from the methyl proton e in the spectrum of xanthogallin.

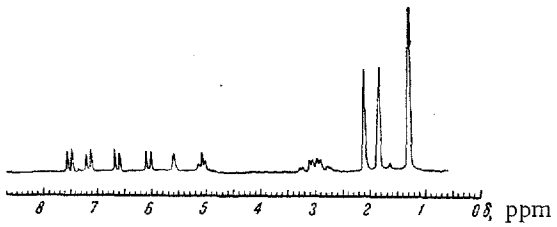
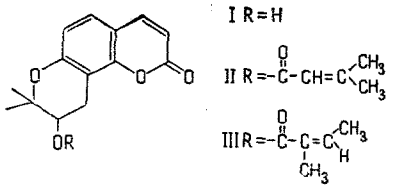


Fig. 4. NMR spectrum of buchtormin in CCl_4

Attempts to separate the buchtormin and xanthogallin by paper and thin-layer chromatography using various systems of solvents were unsuccessful, in view of the closeness of the chemical and physical properties of the two compounds.

The structure of buchtormin has been confirmed by its synthesis from xanthogallol and β, β-dimethylacrylyl chloride, kindly provided by V. G. Yashunskii. A pure sample of this compound was obtained in the form of a glassy mass with the comparison $C_{19}H_{20}O_5$, $[\alpha]_D^{20}$ +71.8°. Its UV and IR spectra (Figs. 2 and 3) where typical for dihydropyranocoumarins; the NMR spectrum of buchtormin corresponds completely to the structure proposed for it (Fig. 4).

On the basis of the specific rotations of buchtormin and xanthogallin, the composition of the natural mixture of these compounds was calculated; it was found to contain about 80% of the former and 20% of the latter.



Experimental

The IR spectra were obtained on a UR-10 spectrophotometer. The substances were studied in the form of mulls in paraffin oil. The NMR spectra were recorded on a JNM-4 H-100 (100 MHz) instrument in deuterochloroform and carbon tetrachloride. The positions of the signals were determined relative to tetramethylsilane as internal standard taken as 0.

The paper chromatography was carried out by the ascending method using petroleum ether (bp 40–60° C) as solvent.

The paper was impregnated with a solution of formamide in acetone (1 : 3) [3]. The revealing agent was Pauly's reagent according to Kutáček [7]. The thin-layer chromatography (TLC) was carried out on plates with a nonfixed layer of neutral alumina (Brockmann and Schodder activity III) [8].

The following mixtures were used as solvent systems: 1) n-hexane—diethyl ether (1 : 1), and 2) benzene—butyl acetate (2 : 1). The positions of the spots were determined by their fluorescence in UV light.

Bergapten and isoimperatorin. One kilogram of the roots was extracted with methanol. The extract was concentrated to 600 ml, diluted with an equal volume of water, and shaken with 2 *l* of ether. The ethereal extract was treated with 0.5% caustic potash solution, washed with water, and dried with anhydrous sodium sulfate, and the solvent was distilled off. This gave 30 g of a resinous residue. Fifteen grams of this resin was chromatographed on a column of alumina (800 g, acetic, activity II, 24 × 7 cm). Elution was carried out with a mixture of benzene and petroleum ether (bp 40–60° C) (2 : 1), with benzene, and with diethyl ether. On standing, the 7-th and 8-th fractions deposited a small amount of crystals with mp 186–187° C and R_f 0.47 on paper and with an IR spectrum corresponding to that of bergapten.

Isoimperatorin was obtained from the 9-th and 10-th fractions in the form of needles melting at 108–109° C after recrystallization from methanol, R_f 0.65 (paper chromatography) and 0.5 (TLC). A mixture with an authentic sample gave no depression of the melting point. The IR spectrum was identical with that of isoimperatorin.

Xanthogallol. Two kilograms of the comminuted roots was extracted with petroleum ether. The raw material freed from essential and fatty oils was extracted three times with diethyl ether. The resin (28.1 g) obtained after the evaporation of the solvent was chromatographed on a column of alumina (2 kg, neutral, activity III, 80 × 6 cm). Elution with a system of solvents having increasing polarity gave 48 fractions. The fractions chromatographically (TLC) similar in composition were combined and were re-separated on a column of the same adsorbent in a ratio of 1 : 50. A mixture containing a substance with Rf 0.30 (TLC, system 2) on elution with petroleum ether and chloroform (2 : 1) gave needles with mp 182–183° C which, after recrystallization from ethanol, melted at 184–185° C, $[\alpha]_D^{19}$ +14° (c 2.0; chloroform). The substance was shown to be identical with xanthogallol by its IR spectrum and a mixed melting point.

Substance (II). The combined fractions containing mainly the component with R_f 0.35 (TLC, system 1) was dissolved in chloroform and separated by means of preparative thin-layer chromatography on plates with a nonfixed layer of alumina (activity III, 32 × 32 cm) in the benzene–methanol (9 : 1) system. The zones collected individually on the basis of the nature of their fluorescence were exhaustively extracted with ether or methanol.

The yellow resin obtained after the evaporation of the solvent from the fractions corresponding to substance (II) were chromatographed under the given conditions in the benzene–methanol (98.5 : 1.5) system. After three purifications by this method, a chromatographically homogeneous substance was obtained in the form of a colorless vitreous mass $[\alpha]_D^{13}$ +50° (c 2.0; chloroform). This substance was also isolated by direct thin-layer chromatography of the ethereal extract.

Found, %: C 69.26, 69.48; H 6.35, 6.35. Calculated for $C_{19}H_{20}O_5$, %: C 69.46; H 6.14.

Hydrolysis of substance (II). Three grams of the substance was heated with 50 ml of a 10% solution of caustic potash in methanol in the water bath for 2 hr. The cooled mixture was diluted with water (100 ml) and the methanol was distilled off; the alkaline aqueous solution was treated with ether and was then acidified with 20% sulfuric acid and extracted with chloroform. The organic layer was successively shaken with portions of 10% ammonia solution, washed to neutrality with water, dried with anhydrous sodium sulfate, and evaporated. The product (1.9 g), when recrystallized from ethanol, gave snow-white needles with mp 184–185° C, $[\alpha]_D^{18}$ +8° (c 5.0; chloroform).

The IR spectrum of the substance obtained was identical with that of substance (I) (xanthogallol). A mixture with an authentic sample gave no depression of the melting point.

Acetate of the hydroxylactone. 0.2 g of the substance was heated for 1 hr with 2 ml of a mixture of acetic anhydride and pyridine (1 : 1) in the water bath. After the elimination of the solvent and recrystallization from ethanol, needles were obtained with mp 138.5–140° C, $[\alpha]_D^{20}$ +2.5° (c 2.0; chloroform).

Found, %: C 66.76, 66.85; H 5.68, 5.87. Calculated for $C_{16}H_{16}O_5$, %: C 66.64; H 5.59.

Synthesis of xanthogallol β,β-dimethylacrylate. A mixture of 0.95 g of β,β-dimethylacrylyl chloride and 2.04 g of xanthogallol in 20 ml of dry benzene was heated for 27 hr, the course of the reaction being followed by TLC. On cooling, the mixture deposited crystals of unchanged xanthogallol which were filtered off (0.6 g). The filtrate was evaporated in vacuum. The resinous residue (2.2 g) was chromatographed on a column of alumina (100 g, activity II). Elution with a mixture of n-hexane and chloroform (4 : 1) gave a vitreous mass (0.92 g) having on a chromatogram (TLC, system 2) a R_f value corresponding to buchtormin (0.75), $[\alpha]_D^{20}$ + 71.8° (c 4.11; chloroform).

Found, %: C 69.46, 69.73; H 6.16, 6.19. Calculated for $C_{19}H_{20}O_5$, %: C 69.46; H 6.14.

Conclusions

From the roots of Libanotis buchtormensis (Fisch.) D. C. have been isolated the furocoumarins bergapten, isoimperatorin, a pyranocoumarin, xanthogallol, and a new coumarin buchtormin $C_{19}H_{20}O_5$, for which the structure (+)-3'-(β,β-dimethylacryloxy)-2',2'-dimethyl-3',4'-dihydropyrano-5',6':8,7-coumarin has been proposed and confirmed by synthesis.

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27 March 1967

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