A NEW ACID FROM THE OIL OF THE SEEDS OF *Sambucus nigra*

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In a study of the fatty-acid composition of the oil of the seeds of Sambucus nigra, family Caprifoliaceae, we detected [1] an acid (I) the position of which on paper chromatography in the acetic acid (98%)–formic acid (85%)–water (75:25:2.5) system [2] corresponded to R_f 0.98.

After isolation by column chromatography and purification by TLC, it consisted of a viscous yellow liquid insoluble in water and petroleum ether, sparingly soluble in carbon tetrachloride, and very readily soluble in diethyl ether, ethanol, and other polar solvents. It has the composition $C_{18}H_{30}O_6$, $[\alpha]_D^{20}$ −32.4° (0.2; ethanol), n_D^{24} 1.4870, d_{24}^{24} 1.0530, MR_D 93, calculated from the atomic refractions taking into account the exaltation of the bonds in an open chain 91, mol. wt. 342.9 (from the neutralization number).

The acid (I) forms water-soluble soaps and reacts with carbonyl reagents, including the giving of a positive reaction for an α-diketo group [3] and its trans-enolic form ([4, 5], pp. 375, 377). Substance (I) decolorizes a 2% solution of bromine, but it does not undergo hydrogenation under either mild or severe conditions.

The iodine number (I. No.) of (I) is 24.2 (% I_2), in contrast to the theoretically calculated figure of 74.2 for one double bond, which confirms the absence of a double bond.

The IR spectrum of (I) (Fig. 1, curve I) shows absorption bands at (cm^{-1}) 1280, 1250, 1190, 1080, 1050 (carboxy group), and 3400-2500 (dimer of an acid) in the form of a broad band of medium intensity.

The integral intensity of the carbonyl band is twice that for stearic acid. This enables (I) to be assigned to the group of oxo carboxylic acids.

In addition, the spectrum has bands at (cm^{-1}) 1640 and 970 (trans-enolic bond), 1100 (secondary hydroxyl), 2850, 1460, and 725 (aliphatic chain), and 2920, 1410, and 1380 (methyl group).

The NMR spectrum of (I) (Fig. 2) has a triplet *a* at 0.8 ppm, J = 13 Hz (~ 3 H) and a poorly-resolved quartet d at 2.0 ppm, J = 13 Hz (2 H). The chemical shift (CS) of the quartet d shows that in the acid an ethyl group is adjacent to the carbonyl and the stronger deshielding of its methylene protons at 2.0 ppm as compared with the α-protons for an isolated carbonyl group (~ 1.5 ppm) determines the position of the α-diketo group at C_{15} and C_{16}. The "methylene peak" b at 1.2 ppm (14 H) shows the presence in (I) of seven methylene groups not adjacent to a carbonyl group.

The triplet e of the α-methylene groups 2 and 14 (adjacent to the carboxy and to the α-diketo group) is located at 2.3 ppm (4 H). A multiplet g of the methine proton of the secondary alcohol group is found at 3.5 ppm (~ 1 H), which is confirmed by its paramagnetic shift in the spectrum of the acetate to 4.8 ppm (1 H). The NMR spectrum of (I) shows diffuse signals h at 4.0 ppm, J = 15 Hz, i at 5.75 ppm, J = 20 Hz, of the methine protons at C_{17} and C_{14} of the enolic forms of the α-diketo group, and j at 7.4 ppm, J = 40 Hz, of the enolic hydroxyls of (II) and (III). The broad diffuse form of the signals of the enolic protons of the α-diketo group is due to the averaging of the CSs of the two unsymmetrical tautomeric enols (II) and (III).

In the UV spectrum of (I) there is absorption at λ_{max} 230 and 280 nm (log ε 4.85; 2.9), which is typical for the enolic form of an α-diketo group. According to the I. No., the amount of enol in (I) is ~ 30%.

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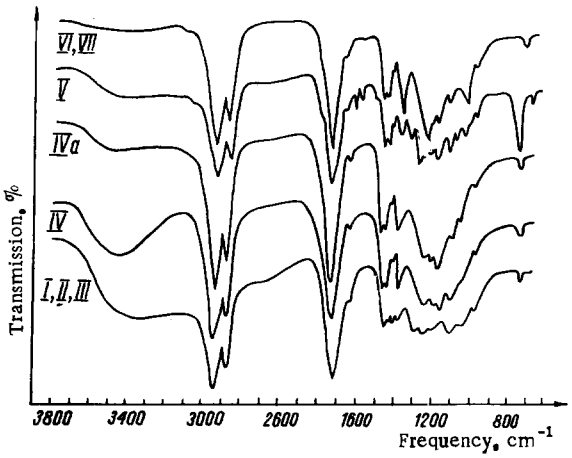


Fig. 1. IR spectrum of the oxo acid (I) [keto–enol mixture with 30% of the enolic tautomers (II) and (III)]; the methylene ester (IV) and (IVa) (in the form of a dilute solution in carbon tetrachloride); the benzoate of the methyl ester (V); and the diacetate of the methyl ester (mixture of derivatives of the tautomeric enols) (VI) and (VII).

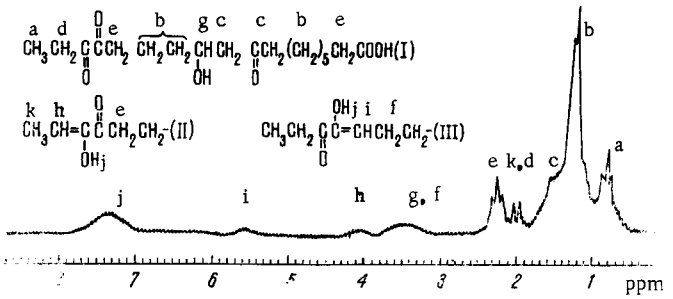


Fig. 2. NMR spectra of the oxo hydroxy acid (I) [with 30% of the enolic tautomers (II) and (III)].

The esterification of (I) with diazomethane gave the ester (IV) with mol. wt. 356 (mass spectrum). The formation of an ester group is confirmed by the disappearance of IR absorption in the 3400-2500 and 1280 cm^{-1} regions (Fig. 1, IV), by the appearance at 3.5 ppm of a singlet of four protons, of which one is a methine proton (—CH—) and three are methoxy protons, and by the negative reaction with copper acetate.

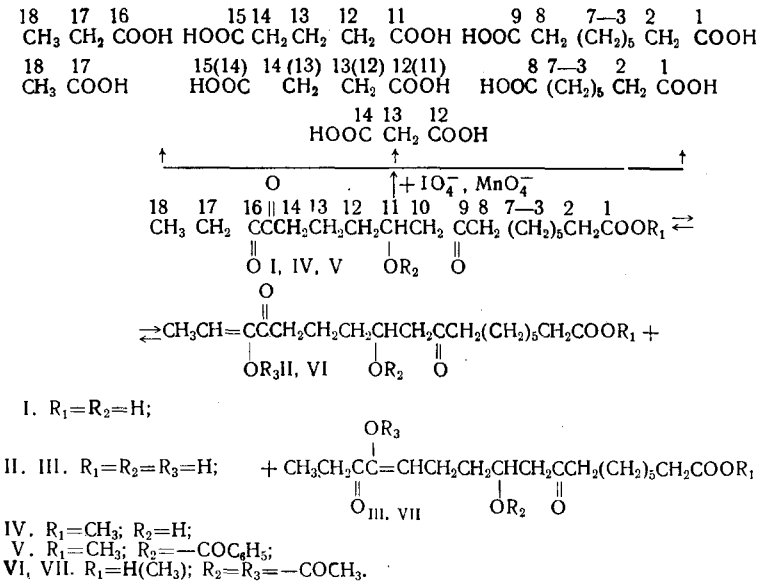
OH

The iodine number of (IV) of about 3 corresponds to 4% of enol in the keto–enol mixture. The presence in the IR spectrum of (IV) of a broad band at 3600-3300 and a band at 1020 (or 1070) cm^{-1} shows the presence in the substance of a hydroxy group involved in a hydrogen bond.

The presence of a secondary alcohol group is shown by the formation of the monobenzoate of the methyl ester (V), in the IR spectrum of which (Fig. 1, V) the band of the secondary hydroxyl at 1100 cm^{-1} has disappeared and there is the absorption of ester bonds at 1125 and 1280 cm^{-1}. Two multiplets (5 H) of a benzoyl group have appeared in the NMR spectrum in the 8.1 and 7.5 ppm regions.

The acetylation of (I) with acetic anhydride gave a mixture of the diacetates (VI) and (VII) corresponding to the tautomeric enols (II) and (III). As is well-known ([4], p. 525), an α-diketo group acetylates readily. In actual fact, in the IR spectrum of the diacetates (Fig. 1, VI and VII) the region of the absorption of the hydroxyls has disappeared. The acetyl number and a six-proton peak at 1.9 ppm in the NMR spectrum of (VI) and (VII) show the presence of two acetoxy groups in them. Furthermore, in the UV spectrum of the diace-

tates in the presence of alkali there is a bathochromic shift of 16 nm (230 → 246 nm), which also confirms the acetylation of the α-diketo group ([4], p. 128).



A direct comparison of the areas of the multiplet signals of the isolated protons [7] in the 4.8 and 5.3 ppm regions shows that the amount of (VII) is 46% of the total of the two diacetyl derivatives of (I). Consequently, the enolization of the two α-carbonyls at C_{15} and C_{16} of (I) is equiprobable.

The composition of the 2,4-dinitrophenylhydrazone (DNPH) (VIII) corresponds to the addition of two molecules of 2,4-dinitrophenylhydrazine. This is confirmed by the presence in the molecule of (I) of one isolated carbonyl and one α-diketo group, since under the reaction conditions without heating only one molecule of 2,4-dinitrophenylhydrazine adds to the latter ([5], p. 465). The signal of the =C=N group in the UV spectrum of (VIII) is found at λ_{max} 367 nm (log ε 4.02). The absorption of the $N=\overset{|}{C}-\overset{|}{C}=O$ group obviously shifts into the visible region because of the conjugation of the two chromophores. The methyl ester of (I) forms solid derivatives at the α-diketo group – a nickel dioximate and a quinoxaline derivative.

To determine the position of localization of the functional groups, compound (I) was subjected to periodate–permanganate degradation. Among the dicarboxylic oxidation products were found predominating amounts of azelaic and glutaric acids, smaller amounts of suberic and succinic acids, and traces of malonic acid, while the monocarboxylic products included acetic and propionic acids.

When (IV) was oxidized, the main degradation product found was the semiester of azelaic acid, which was identified by TLC in comparison with the semiester of azelaic acid obtained by the oxidation of methyl oleate. This shows, in the first place, the position of an aliphatic chain with seven methylene groups at the carboxyl end of (I) and, in the second place, the predominant cleavage of the 9–10 bond.

The formation of two dicarboxylic acids – azelaic and suberic – corresponds to the cleavage of the 9–8 and 9–10 bonds. We have shown previously [8] that such cleavage on periodate–permanganate degradation takes place with the partial oxidation of the carbon atom. According to these facts, C_9 bears an oxygen-containing group.

With 18 carbon atoms in the molecule of (I), the monocarboxylic products – acetic and propionic acids – can relate only to the methyl end of the chain $CH_3CH_2-\overset{|}{C}=$, in which C_{16} is also partially oxidized.

Thus, from the results of oxidative degradation it is possible to assume the localization of the oxygen-containing groups at C_1 (–COOH), C_9, C_{16}, and in the C_{10}-C_{15} section of the chain. As shown above, the position of the α-diketo group has been established at C_{15} and C_{16} on the basis of the NMR spectrum of (I).

In agreement with this, the predominance of glutamic acid among the low-molecular-weight dicarboxylic oxidation products unambiguously determines the last of the functional groups at C_{11}. Consequently, the isolated carbonyl and the secondary hydroxyl are located either at C_9 and C_{11} or at C_{11} and C_9, respectively.

The β position of these groups is confirmed by the formation of a β diketone on the oxidation of (IV) with chromium trioxide, by the existence of an intramolecular hydrogen bond [IR spectra of (IV) and (IVa), Fig. 1], by the halving of the integral intensity of the carbonyl band of (I) (which is also connected with the keto–enol exchange of the α-diketo group), and by the shift in the singlet signal of the secondary hydroxyl into a very weak field beyond the limits of the NMR spectrum.

The characteristic reaction of (I) with salicylaldehyde ([5], p. 436) enables the isolated carbonyl to be placed at C_9. The mass spectra of (I) and (V) confirmed this suggestion. Otherwise, the series of fragments with m/e 67 (41%), 81 (58%), and 95 (59%) in the spectrum of (I) could not have been explained, and the fragments with m/e 152 (11%) and 185 (20%) would have been absent from the spectrum of (V).

In addition to the fragments mentioned, the mass spectrum of (I) has a number of characteristic peaks with m/e 55 (100%), 57 (67%), 59 (32%), 69 (87%), 71 (57%), 74 (79%), 83 (88%), 85 (43%), 87 (92%), 97 (62%), 111 (37%), 125 (20%), 127 (25%), 129 (35%), 141 (28%), 143 (31%), 155 (51%), 156 (20%), 157 (20%), 171 (20%), 185 (32%), 197 (49%), 199 (20%), 200 (20%), 225 (12%), 229 (29%), 253 (10%), and 256 (15%), $[M-85]^+$ (19%), $[M-43]^+$ (13%), $[M-15]^+$ (18%), $[M-1]^+$ (8%), and M^+ (3%), confirming the presence of five centers initiating the decomposition of the molecular of (I) in the suggested positions of localization of the oxygen-containing functional groups.

Thus, on the basis of the results obtained, structural formula (I) may be proposed for the new keto hydroxy acid, which we have called caprifolonic acid.

EXPERIMENTAL

Chromatography was performed on grade "M" ["slow"] paper of the Leningrad No. 2 mill impregnated with a 10% solution of paraffin oil in benzene; KSK silica gel (100 mesh for column chromatography and 150 mesh for thin-layer chromatography) was washed with hydrochloric acid, water, acetone, methanol, and chloroform. The composition of the fatty acids was determined by the gas-chromatographic method on a UKh-2 chromatograph at 200°C with a column 2.5 m long. Poly(ethylene succinate) was used as the stationary phase.

The IR spectra were taken on a UR-10 instrument in a thin layer, the UV spectra on a Hitachi spectrophotometer in ethanol in concentrations of 0.1-0.2 mg/ml, and the mass spectra on a standard MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source at 100°C at an ionizing voltage of 40 V.

The NMR spectra were obtained on a JNM-4H-100/100 MHz instrument at room temperature with concentrations of 5-10% in carbon tetrachloride using HMDS as internal standard. (The chemical shifts are given on the δ scale.)

The elementary analyses of the compounds corresponded to the calculated figures. The melting points are uncorrected.

The isolation of (I) was performed by column chromatography on silica gel [7] with additional purification by TLC on silica gel in the petroleum ether–diethyl ether–acetic acid (9 : 1 : 0.1) system [9]. The individuality of (I) was checked by the same method in the petroleum ether (40-60°C)–diethyl ether (60 : 40) system [10], which separates hydroxy acids according to the number of hydroxy groups. Yield 2% (from the mixture with fatty acids).

Hydrogenation. The oil and the mixture of acids obtained from it were exhaustively hydrogenated over a palladium–aluminum catalyst in acetic acid solution at 60°C and over a Raney Ni catalyst at 230-240°C, respectively. Substance (I) was isolated from the hydrogenation products.

Monobenzoate of the Methyl Ester (V). A mixture of equimolar (0.2 mmole) amounts of (IV) and benzoyl chloride was kept at room temperature for 48 h and then in the vacuum-drying chest at 50°C for 4 h. The yield of (V) was 99% (of theoretical).

The Diacetates (VI) and (VII). Acetylation was performed by the usual method ([5], p. 318) in an excess of acetic anhydride with the addition of freshly fused sodium acetate. The mixture was boiled for 3 h. The yield of the mixture of (VI) and (VII) was 80% (of theoretical). Acetyl No. 131.2 and mol. wt. 425.3.

2,4-DNPH (VIII). The reaction was performed with an aqueous ethanolic solution of 2,4-dinitrophenylhydrazine in admixture with phosphoric acid ([5], p. 443).

The individuality of (VIII) was confirmed by TLC on silica gel in the ethyl formate–petroleum ether (40-60°C)–acetic acid (50:50:1) system [11], which separates the DNPHs of oxo acids according to the position of the carbonyl group relative to the carboxyl.

Nickel Dioximate (IX). The reaction of (IV) with hydroxylamine in the presence of nickel chloride was performed by the usual method ([5], pp. 455, 469). The yield of product was 97% (of theoretical).

Quinoxaline (X). Equimolecular amounts of (IV) and of o-phenylene diamine (0.2 mmole) were heated in a conical test-tube in the boiling water bath for 1 h. The solid product obtained was washed with water three times and was dried in vacuum at 60°C for 6 h. Then it was treated three times with diethyl ether and dried under the same conditions. This gave 87% (of theoretical) of a brown-red powder with mp 136-137.5°C (dilute ethanol).

Oxidation of (I) and (IV) and of Methyl Oleate [8]. The mixture obtained by periodate–permanganate degradation was identified by the GLC and TLC methods with standard samples. The methyl esters of the acids were identified by comparing the retention times of the peaks of the substances under investigation and of a control mixture of standard samples and by means of a graph of the dependence of the logarithms of the retention times of the components on the number of carbon atoms in the molecules of the acids. In addition, standard samples of the presumable components were introduced into the mixture studied both in GLC and in TLC.

SUMMARY

A new keto hydroxy acid (I) $C_{18}H_{30}O_6$ has been isolated from the fatty oil of the seeds of Sambucus nigra in an amount of 2% of the total fatty acids. A study of its chemical properties and spectral characteristics has shown that it is probably 11-hydroxy-9,15,16-trioxooctadeconoic acid, and that it consists of a keto–enol mixture containing about 30% of the two equiprobable tautomers (II and III); it has been given the name of caprifolonic acid.

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