SEED OIL OF *Artemisia absinthium*

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The oil of the seeds of *Artemisia absinthium* has yielded 95.5% of glycerides. Of these, 59.5% consists of unoxidized and 32.0% of oxidized triglycerides, 1.3% of diglycerides, and 2.7% of monoglycerides. The remaining 4.5% of the oil is made up of hydrocarbons, sterols, sterol acetates, and free fatty acids. The oxy acid content has been calculated from the results obtained (∿12% of the total fatty acids of the oil) and this figure is close to that for the oxy acid content found from the neutralization number of the mixture of fatty acids of the oil (∿11%).

About 15% of epoxy acids and about 8% of α-hydroxy dienoic acids with conjugated ethylenic bonds have been found in the seed oil of *Artemisia absinthium* L. (common wormwood), family Compositae [1, 2]. However, the triglycerides containing these oxidized fatty acids have not been isolated and their structures have not been studied. The seed oil of this plant growing in Central Asia has not been studied, either. In view of this, to isolate the oxidized triglycerides and to obtain model samples of oxy acids we have studied the seed oil of the common wormwood collected on the slopes of the Western Tien-Shan in August-September. The main indices of the seeds and oil were as follows:

Weight of 1000 seeds, g	0.056
Petroleum ether extract calculated on the absolutely dry substance, %	31.46
Density d_4^{20}	0.9216
Refractive index n^{20}	1.4775
Saponification No., mg KOH/g	191.42
Hehner No., %	89.25
Acid No., mg KOH/g	6.37
Iodine No., % I_2	135.42
Acetyl No., mg KOH/g	110.90
"Unsaponifiables" content, %	3.54
Phosphatides content, %	0.18

Below we give the composition of the fatty acids of the oil (mole % on the total):

14:0	0.2
16:0	5.8
$16:1^{9}$	1.0
18:0	0.8
$18:1^{9}$	13.2
$18:2^{9,12}$	67.2
Epoxy-(12:13)-$18:1^{9}$	1.3
Epoxy-(9:10)--$18:1^{12}$	5.0
Epoxy-(9:10)--18:0	Tr.
Hydroxy-18:2	5.5
Neutralization No. of the total fatty acids of the oil, mg KOH/g	199.97

The IR and NMR spectra showed that the main components of the oil were fatty acid (FA) glycerides. The UV spectrum of the oil contained the absorption of conjugated ethylenic bonds (231 nm). After boiling in glacial acetic acid, the oil began to absorb at 261 271,

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TABLE 1. Composition of the Oil with Respect to Classes of Lipids (Column Chromatography)

Lipids		Composition of the fatty acids, % of total						
Class	% of total	16:0	18:0	18:1	18:2	18:3	epoxy 18:1	hydroxy-18:2
Carbohydrates*	0,2	—	—	—	—	—	—	—
Esters*	Traces	—	—	—	—	—	—	—
Triglycerides of u-FAs	59,5	5,4	0,5	13,0	80,2	—	—	—
Monoepoxyacyl triglycerides	13,7	5,6	6,6	9,7	50,3	—	33,3	—
Free u-FAs	3,3	12,1	3,3	22,6	60,7	1,3	—	—
Monohydroxyacyl triglycerides	11,4	5,2	0,5	10,0	50,9	—	—	33,3
Di(epoxy-hydroxy)acyl triglycerides	3,2	4,1	1,3	7,2	20,8	—	33,3	33,3
Diglycerides†	1,3	30,1	4,1	20,8	37,8	—	—	—
Free sterols*	0,4	—	—	—	—	—	—	—
Oxidized triglycerides-1	2,5	7,80	0,9	15,3	76,0	—	?	?
Oxidized triglycerides-2	1,2	10,8	2,1	21,3	65,8	—	?	?
Free o-FAs	0,6	—	—	—	—	—		100‡
β-Monoglycerides	1,7	11,8	4,0	18,3	63,5	2,4	—	—
α-Monoglycerides	1,0	12,2	2,3	17,3	68,2	—	—	—

*See Experimental part.

†The following acids were found in the diglycerides in addition to those given in the table: 10-iso (1.6%), 11:0 (0.8%); 13:0 (0.7%), 14:0 (0.7%), and 16-iso (3.4%).

‡Total of epoxy and hydroxy acids.

and 282 nm. This shows the appearance of conjugated trienic systems, which can be formed as the result of the dehydration of α-hydroxy dienic systems with two conjugated ethylenic bonds.

By alkaline hydrolysis, a mixture of FAs was isolated from the oil, with the separation of the "unsaponifiables." This mixture absorbed in the same way as the oil, and on a paper chromatogram (PC) hydroxy acids with R_f 0.93 were detected.

The mixture of FAs was methylated with diazomethane. The IR spectrum of the mixture of FA methyl esters obtained showed the absorption bands characteristic for OH groups.

The mixtures of FA methyl esters was separated into oxidized FAs (o-FAs) and unoxidized FAs (u-FAs) by column chromatography in the hexane–diethyl ether (9:1) system.

After the o-FAs has been boiled in glacial acetic acid, octadecatrienoic acids were formed which, on hydrogenation, were converted into steric acid (PC); this shows the presence of α-hydroxy dienic systems in the structure of the o-FAs [3-5].

By transesterification with methanol using sodium methanolate as catalyst, a mixture of FA methyl esters was obtained from the oil, and from these by column chromatography we isolated the methyl esters of the trans isomers of 9-hydroxy, 11-hydroxy-, and 13-hydroxyoctadecadienoic acids, and also of cis-12,13-epoxyoctadec-cis-9-enoic, cis-9,10-epoxyoctadec-cis-12-enoic, and (in traces) 9,10-epoxyoctadecanoic acids.

The mixture of u-FA methyl esters was separated on a "silver" column [6] according to their degrees of unsaturation. Three fractions of methyl esters were obtained — "saturated," "monoenic," and "dienic." According to gas–liquid chromatography (GLC), the first of them contained methyl esters of the 16:0, 18:0, and, in traces, 20:0 and 22:0 acids. The methyl esters of the acids from the other two fractions were subjected to oxidative degradation by Rudloff's method with periodate-permanganate. The degradation fragments, after methylation with diazomethane, were analyzed by the GLC method. In the degradation products of the "monoenic" esters were found the methyl esters of nonane-1,9-dicarboxylic, n-nonane, and, in traces, n-heptanoic acids, and in the products of the degradation of the "dienic" esters, the methyl esters of nonane-1,9-dicarboxylic and n-hexanoic acids. The results obtained showed that the monoenic and dienic acids of the oil consisted of the widely distributed acids oleic and linoleic, respectively. The composition of the u-FAs, according to GLC, was as follows (mol.%): 14:0 — 0.2; 16:0 — 6.5; 16:1 — 1.1; 18:0 — 0.8; 18:1 — 15.1; 18:2 — 76.3.

Making use, as we have shown previously [5], of the neutralization numbers, we calculated the content of o-FAs as ∿11% of the mixture with the u-FAs.

To determine the amount of individual classes of lipids, the oil was subjected to column chromatography and the individual fractions were studied as described previously [7]. The results (Table 1) showed that the amount of o-FAs in the total mixture of FAs of the oil was ~12%. This agrees with the figure obtained from neutralization numbers. Furthermore, it follows from Table 1 that there were 6.3% of epoxy-FAs and 5.5% of hydroxy-FAs, of the total amount of acids of the oil, i.e., the ratio between the epoxy- and hydroxy-FAs was close to 1:1. This ratio does not correspond to that found from the degradation fragments and from the results of the UV spectroscopy of the methyl esters of the corresponding acids isolated by the transesterification of the oil and the column chromatography of the methyl esters [5]. They obviously change in the process of isolation because of the lability of the hydroxy acids. The results obtained show the absence of the signals of epoxide rings in the NMR spectrum of the oil, since detection by the NMR method is effective only when the concentration of epoxy acids is greater than 5-6% [8].

We calculated the total fatty acid composition of the oil on the basis of results on the amount of o-FAs and the ratio of the epoxy acids (see above). The IR spectra of the hydroxyacyl triglycerides showed no transethylenic bonds, the IR spectra of the oxidized fatty acids isolated from them by alkaline hydrolysis showed bands (990 and 950 cm^{-1}) corresponding to cis-trans conjugated double bonds. After the separation of the total FA methyl esters only trans-trans conjugation was found. Consequently, in the process of isolating and separating the FAs a rearrangement takes place with the formation of the thermodynamically more stable trans,trans-conjugated dienic systems.

The fatty-acid compositions of the triglycerides isolated, found by the GLC of the corresponding methyl esters, are given in Table 1, and the position-type compositions calculated by Coleman's method in Table 2, where the fatty acid radicals are combined into the following groups: P) 14:0 + 16:0 + 18:0; O) 16:1 + 18:1; L) 18.2; E) e-18:1; H) h-18:2.

It follows from Table 1 that the only difference between the compositions of the FAs of the unoxidized and the oxidized triglycerides consists in the linoleic acid content. While in the unoxidized triglycerides it is about 80%, in the oxidized triglycerides this figure corresponds to the sum of the amounts of linolenic and of oxidized acids, which shows the predominant oxidation of the linoleic acid.

The compositions of the free FAs and of the FAs of the diglycerides differ sharply from the composition of the FAs of the triglycerides. Consequently, the first two are not decomposition products of the triglycerides. 1(3)- and 2-monoglycerides and free FAs are more frequently found to be biogenetically linked, having similar FA compositions.

It can be seen from Table 2 that 83.5% of the epoxyacyl radicals in the monoepoxyacyl triglycerides are present in positions 1 and 3 and 50% of the hydroxyacyl radicals in the monohydroxyacyl triglycerides occupy these positions. Among the di(epoxy-hydroxy)acyl triglycerides there are 50% of types in which both oxidized radicals are present in the extreme positions, the other 50% of types having oxidized radicals in positions 1 and 2. The highest amount is represented by those oxidized triglycerides in which the acyl radicals are the most unsaturated. This also indicates that the oxidized triglycerides are formed from the unoxidized triglycerides through the oxidation of the linoleyl radical.

EXPERIMENTAL

The isolation of the oil from the seeds, the spectral analyses, column and gas–liquid chromatography, alkaline hydrolysis, and oxidative degradation were all carried out as described previously [5]. The indices of the oil were determined by handbook methods [9].

Hydrocarbons were identified by analytical thin-layer chromatography (Silufol, hexane, R_f 0.98, revealed in iodine vapor), on the basis of their IR spectra (ν_{max}^{film}, cm^{-1}: 2965 s, 2880 s; 1380 m, $-CH_3$; 2935 v.s, 2885 v.s, 1465 s, 725, 735 s, $-CH_2-$), and by mass spectrometry. Mass spectrum at 20-25°C, 40 V, m/e (%): M^+ 352 (1), 350, 348, 346, 344 (0.6) – C_{25}; M^+ 336, 334, 332, 330 (1.5) – C_{24}; M^+ 322, 320, 318, 316 (3.3) – C_{23}; M^+ 308, 306, 304, 302 (4.2) – C_{22}; M^+ 294, 292, 290, 288 (6.0) – C_{21}; M^+ 266, 264, 262, 260 (6.0) – C_{20}; 309, 307, 305, 303, 301 (1.0); 295-285 (5.0); 281-273 (5.0); 267-259 (6.0); 253-245 (6.6); 239-231 (6.0); 225-217 (7.8); 211-203 (8.4); 197-189 (12.9); 183-175 (15.0); 169-161 (18.0); 155-147 (25.0); 141-133 (22.0); 125-121 (30.6); 111-109 (50.0); 97-95 (90.0); 85-81 (84.6); 71-69 (100.0); 57 (30.0); 55 (90.0); 43 (73.6); 41 (25.0). Mass spectrum with full-sample admission, 130°C, 17 V, m/e (%): M^+ 436 (13.0); M^+ 422 (4.0); M^+ 408 (40.0); M^+ 394 (8.0); M^+ 380 (43.0); M^+ 366 (22.0); M^+ 352 (20.0); 421 (4.0); 407 (8.0); 393 (8.5); 379 (20.0); 365

TABLE 2. Position-Type Composition of the Triglycerides of the Oil, mol. %

Triglycerides of u-FAs		Monoepoxyacyl triglycerides		Monohydroxyazyl triglycerides		Di(epoxy-hydroxy)acyl triglycerides	
type	content	type	content	type	content	type	content
PPL	0,1	PPE	0,1	PPH	0,1	EPH	0,7
OPO	0,3	OPE	—	OPH	0,1	HPE	0,7
OPL	1,2	LPE	0,2	LPH	0,2	EOH	4,3
LPO	1,2	EPP	0,1	HPP	0,1	HOE	4,3
LPL	5,7	EPO	—	HPO	0,1	ELH	20,0
POL	0,1	EPL	0,2	HPL	0,2	HLE	20,0
OOO	0,4	POE	1,0	POH	0,1	PHE	3,7
OOL	1,7	OOE	1,0	OOH	0,1	PEH	3,7
LOP	0,1	LOE	3,7	LOH	0,3	OEH	3,2
LOO	1,7	EOP	1,0	HOP	0,1	OHE	3,2
LOL	8,2	EOO	0,8	HOO	0,1	LHE	5,3
PLO	0,1	EOL	3,7	HOL	0,3	LEH	5,3
PLL	0,5	PLE	6,3	PLH	2,4	EHP	3,7
OLP	0,1	OLE	5,3	OLH	8,7	HEP	3,7
OLO	2,4	LLE	24,3	LLH	15,2	EHO	3,2
OLL	11,3	ELP	6,2	HLP	2,3	HEO	3,2
LLP	0,4	ELO	5,5	HLO	4,3	EHL	5,9
LLO	11,3	PEP	0,6	PHP	0,5	HEL	5,9
LLL	53,2	PEO	0,6	PHO	1,1	—	—
—	—	PEL	1,7	PHL	3,9	—	—
—	—	OEP	0,6	OHP	1,1	—	—
—	—	OEO	0,6	OHO	1,9	—	—
—	—	OEL	1,5	OHL	6,8	—	—
—	—	LEP	1,7	LHP	3,9	—	—
—	—	LEO	1,7	LHO	6,6	—	—
—	—	LEL	7,6	LHL	24,2	—	—
—	—	ELL	24,1	HLL	15,3	—	—

(25.0); 351 (27.0); 337 (31.0); 323 (35.0); 309 (40.0); 295 (45.0); 281 (46.0); 267 (45.0); 253 (48.0); 239 (51.0); 225 (56.0); 211 (58.0); 197 (60.0); 183 (65.0); 169 (72.0); 155 (76.0); 141 (60.0); 127 (68.0); 125 (40.0); 113, 111 (80.0); 99 (100.0); 97 (82.0); 85 (23.0); 83 (22.0); 71 (42.0); 57 (43.0); 43 (9.0); 41 (22.0).

Esters (IR spectra of a film: 1740 v.s, 1270 s, 1175 s, cm^{-1}) having M^+ 456 454, 442, and 428 (mass spectrum, 150°C, 40 V) were acetates of sterols with molecular weights of 414, 412, 400, and 386 present in the oil.

The triglycerides of the u-FAs were transparent in the near ultraviolet and had $[\alpha]_D^{20}$ +0.76° (51.5 mg/ml, in chloroform). NMR spectrum, δ, ppm: t 0.86, m 1.23, m 1.55, d 2.0, t 2.24, m 2.68, m 4.10, m 5.10, m 5.23. IR spectrum, ν_{max}^{film}, cm^{-1}: 3010 m, 1635 w, —CH=CH—; 2970 s, 2880 s, 1380 m, $—CH_3$; 2940 s, 2865 s, 1465 m, 730 m, $—CH_2—$; 1740 s, 1420 m, 1245 s, 1175 s, —OCOR. The composition of the FAs in positions 2 of the triglycerides was determined by enzymatic hydrolysis with pancreatic lipase [7]. The 2-monoglycerides were hydrolyzed with alkali. The composition of the FAs of the 2-monoglycerides, according to the GLC of the corresponding methyl esters was as follows (%): 16:0 — 0.7; 18:1 — 17.4; 18:2 — 81.9.

The monoepoxyacyl triglycerides were transparent in the near ultraviolet, $[\alpha]_D^{20}$ −2.38° (52 mg/ml, in chloroform). NMR spectrum, δ, ppm: t 0.86, m 1.30, s 1.55, d 2.02, t 2.24, m combined 2.68 and 2.70 (in the presence of TFA 2.65 and 2.90), m 4.11, m 5.10, m 5.23. IR spectrum, ν_{max}^{film}, cm^{-1}: 3010 m, 2960 s, 2930 s, 2880 s, 2860 s, 1745 s, 1660 w, 1465 m, 1420 m, 1380 m, 1245 s, 1170 s, 845 and 830 m, 730 m. The ratio of the epoxyacyl and other oxidized 2-monoglycerides isolated from the products of enzymatic hydrolysis was 1:5. The composition of the FAs in position 2 according to the GLC of the corresponding MEs was (%): 16:0 — 0.3; 18:1 — 11.3; 18:2 — 71.8; e-18:1 — 16.6.

The free u-FAs, according to the GLC of the MEs obtained from them, had the composition given in Table 1. NMR spectrum of the u-FAs, δ, ppm: t 0.86, m 1.27, m 1.50, d 1.90, t 2.18, m 2.68, s 3.56, m 5.1, m 5.24.

Monohydroxyacyl Triglycerides. UV spectrum, λ_{max} at 233 nm; $[\alpha]_D^{20}$ −17.62° (21.3 mg/ml, in chloroform). IR spectrum, ν_{max}^{film}, cm^{-1}: 3550-3450 m, 3010 m, 2965 s, 2935 s, 2880 s, 2865 s, 1745 s, 1640 w, 1465 m, 1420 m, 1380 m, 1245 s, 1175 s, 1080 s, 990 and 950 m, 730 m.

NMR spectrum, δ, ppm: t 0.86, s 1.26, m 1.56, d 2.00, t 2.22, m 2.68, m 3.51, m 4.10, m 5.10, m 5.23, m 5.5-6.5, s 2-6 (or 7-8 in the presence of TFA).

Among the 2-monoglycerides isolated by enzymatic hydrolysis, there were 50% with hydroxyoctadecadienoyl radicals, the composition of which according to GLC of the corresponding MEs was as follows (%): 16:0 — 0.7; 18:1 — 1.0; 18:2 — 48.3; h-18:2 — 50.

The di(epoxy-hydroxy)acyl triglycerides had maxima at 231 and 234 nm (0.8 D at 0.037 mg/ml). $[\alpha]_D^{20}$ −7.81° (22.4 mg/ml, in chloroform). NMR spectrum, δ, ppm: t 0.85, m 1.28, m 1.55, d 2.01, t 2.22, m combined 2.68 and 2.72, m 3.5, m 4.10, m 5.10, m 5.24, m 5.5-6.5m s 2.0-2.2 (or 7.5 in the presence of TFA). IR spectrum, ν_{max}^{film}, cm^{-1}: 3545-3450, 3015, 2960, 2930, 2880, 2860, 1740, 1645, 1465, 1420, 1380, 1245, 1170, 1110, 990, 950, 840, 830, 730. The ratio of unoxidized, epoxyacyl, and hydroxyacyl 2-monoglycerides as products of enzymatic hydrolysis was 2:1:1. The composition of the FAs of the 2-monoglycerides according to the GLC of the corresponding methyl ester was as follows (%): 16:0 — 1.5; 18:1 — 8.5; 18:2 — 40.0; e-18:1 — 25.0; h-18:2 — 25.0

Diglycerides (sum of the 1,3- and 1,2-isomers). NMR spectrum, δ, ppm: t 0.86, m 1.26, m 1.55, d 2.00, t 2.24, m 2.67, d 3.56, m combined 4.01 and 4.12, m 4.90, m 5.23, s 8.30-8.60 (shifts in the presence of TFA). IR spectrum, ν_{max}^{film}, cm^{-1}: 3500, 3010, 2960, 2930, 2875, 2860, 1745, 1660, 1465, 1420, 1245, 1170, 1060, 730. In the products of alkaline hydrolysis, only the u-FAs the compositions of which are given in Table 1 were found.

Free Sterols. After 10 recrystallizations from methanol and five from acetone, the mixture of sterols was found by mass spectrometry to contain β-sitosterol (M^+ 414) contaminated with α-sitosterol or stigmasterol (M^+ 412), campesterol (M^+ 400), and traces of a sterol with a molecular weight of 386.

The oxidized trigylcerides-1 absorbed at 231 and 233 nm and had $[\alpha]_D^{20}$ +2.6° (19.8 mg/ml, in chloroform). NMR spectrum, δ, ppm: t 0.84, m 1.27, m 1.55, d 1.96, t 2.22, m combined 2.68 and 2.70, m 4.10, m 5.10, m 5.24, m 5.5-6.5. IR spectrum, ν_{max}^{film}, cm^{-1}: 3600-3400, 3015, 2960, 2930, 2880, 2860, 1745, 1725, 1690, 1660, 1465, 1420, 1380, 1240, 1170, 1110, 990, 950, 845, 830, 730. The products of enzymatic hydrolysis were found to contain unoxidized, epoxyacyl, and hydroxyacyl 2-monoglycerides in a ratio of 1.5:1:1. The composition of the FAs in position 2 was as follows (%): 16:0 — 2.3; 18:1 — 7.8; 18:2 — 32.9; e-18:1 — 28.5; h-18:2 — 28.5.

The oxidized triglycerides-2 showed a maximum in the UV spectrum at about 233 nm. The composition of the FAs in position 2 was as follows (%): 16:0 — 2.4; 18:1 — 8.3; 18:2 — 32.3; e-18:1 — 28.5; h-18:2 — 28.5.

The 2-monoglycerides were not cleaved by pancreatic lipase, and alkaline hydrolysis decomposed them with the formation of u-FAs having the composition given in Table 1. NMR spectrum, δ, ppm: t 0.86, m 1.26, m 1.55, d 2.0, t 2.23, m 2.67, d 3.75, m 4.90, m 5.24, s 8.3 (shifts in the presence of TFA). IR spectrum, ν_{max}^{film}, cm^{-1}: 3400, 3010, 2960, 2930, 2885, 2860, 1745, 1650, 1465, 1420, 1380, 1245, 1170, 730.

The 1(3)-monoglycerides were hydrolyzed with pancreatic lipase to form the unoxidized fatty acids the composition of which is given in Table 1. NMR spectrum, δ, ppm: t 0.85, m 1.27, m 1.56, d 1.96, t 2.23, m 2.68, d 3.70, m combined 3.97, m 5.24, m 3.3 (shifts in the presence of TFA). IR spectrum, ν_{max}^{film}, cm^{-1}: 3500, 3015, 2960, 2930, 2875, 2860, 1745, 1660, 1465, 1420, 1380, 1245, 1170, 730.

SUMMARY

The seed oil of *Artemisia absinthium* was found to contain 13 main classes of compounds, according to their amount. Of them eight were glycerides. Five of the triglycerides contained oxy acids, the amount of which was 11-12% of the sum of the fatty acids isolated from the oil.

It has been shown that the amount of hydroxy acids found from the neutralization of the mixed fatty acids of the oil corresponds to the amount of hydroxy acids calculated from the fatty acid compositions of the individual glycerides isolated from the oil.

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TERPENOID COUMARIN GLYCOSIDES OF *Ferula conocaula*

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Two new terpenoid glycoside coumarins have been isolated from the roots of *Ferula conocaula* Korov., and they have been named conferoside and cauferoside. On the basis of the study of chemical and spectral characteristics, their structures have been established as ferocaulinin 4'-O-β-D-glucopyranoside and cauferin 4'-O-β-D-glycopyranoside, respectively.

Continuing a chemical study of the coumarins of *Ferula corocaula* Korov. [1-3], from the water-soluble fraction of an ethanolic extract of the roots we have isolated by chromatography two new coumarin derivatives which we have called conferoside (I) and cauferoside (II).

Conferoside (I) has a composition $C_{30}H_{38}O_{10}$, mp 195-197°C, $[\alpha]_D^{20}$ −110° (c 1.0; ethanol). The UV spectrum of (I) is characteristic for the 7-hydroxycoumarin chromophore. The IR spectrum contains absorption bands due to the presence of an aromatic nucleus, the carbonyl of an α-pyrone, and a hydroxy group.

The acid hydrolysis of conferoside formed umbelliferone and D-glucose, and it is consequently a glycosylated coumarin.

Exhaustive acetylation with acetic anhydride in pyridine led to a tetraacetate of (I) with the composition $C_{38}H_{46}O_{14}$, M^+ 726 (III). The formation of the latter shows that the glycoside is a monoside.

The PMR spectrum of (I) (deuteropyridine) contained the signals from three tertiary methyl groups, 1.04 s, 3 H; 1.29 s, 6 H; from a vinyl methyl group at 1.74 s, 3 H; from the protons of the methylene group in a $-CH_2-O-Ar$ fragment, and also from the protons of a sugar residue at 3.05-5.40 ppm, from an olefinic proton at 5.6 br.s, 1 H, and from the protons of a coumarin nucleus at 6.05-7.70 ppm.

According to its PMR spectrum and its composition, the terpenoid moiety of conferoside must have a bicyclic structure. In actual fact, the enzymatic cleavage of (I) with β-glycosidase [4] gave D-glucose and an aglycone with the composition $C_{24}H_{28}O_5$, M^+ 396. By a comparison of spectral characteristics (IR, NMR) and physical constants, the aglycone of conferoside was identified as ferrocaulinin [1].

The presence in the IR spectrum of (I) of four absorption bands at 1100, 1080, 1040, and 890 cm^{-1} shows that the glucose residue has the pyranose form and is bound by a β-glycosidic bond [5-7]. This was confirmed by enzymatic hydrolysis and by the absolute value of the molecular rotation difference between the glycoside and the aglycone [5]. Thus, conferol is ferocaulinin 4'-O-β-D-glucopyranoside (I).

Cauferoside (II) has the composition $C_{30}H_{40}O_{10}$, mp 176-177°C, $[\alpha]_D^{20}$ −140° (c 1.0; ethanol). Its UV spectrum has the maxima characteristic for 7-hydroxycoumarin. Its IR

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