STRUCTURE OF SIBIRIGENIN

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Khimiya Prirodnykh Soedinenii, Vol. 6, No. 3, pp. 322–328, 1970

UDC 547.918:582.938

As we have previously reported [1], the acid hydrolysis of a mixture of glycosides from Cynanchum sibiricum Willd (family Asclepiadaceae) yielded three separate pregnanes: sibirigenin, penupogenin, and sibicoside. In the present paper we give information on the structure of the least polar of them, sibirigenin.

The elementary analysis of the genin and its molecular weight, determined by mass spectroscopy, correspond to the empirical formula $C_{28}H_{42}O_6$. A maximum in the UV spectrum at 220 mμ (log ε 4.02, ethanol) shows the presence of an α, β-unsaturated carbonyl group. The IR spectrum has absorption bands at (cm^{-1}) 3530, 3345, 1692, 1642–1647 (doublet), 1230, and 1180, showing, respectively, the presence of hydroxyls, a carbonyl group or groups conjugated with a double bond, and an ester group.

The NMR spectrum of sibirigenin clearly shows six three-proton signals in the strong-field region [9.12 (doublet), 8.73, 8.60, 7.90, and 7.83 ppm, τ scale, in C_5D_5N] of which only three (8.73, 8.60, and 7.83) can be ascribed to the pregnane nucleus. Acetylation of the genin under the usual conditions forms a crystalline acetyl derivative in which, judging from the IR spectrum, some of the hydroxyl groups remain unsubstituted. The saponification of sibirigenin with caustic potash in methanol gave an acyclic unsaturated acid with the composition $C_7H_{12}O_2$ and a mixture of neutral products.

The UV spectra of the acid and its methyl ester are similar to the UV spectra of unsaturated carboxylic acids with alkyl substituents in the α, β- or β, β-positions [2]. The mass spectrum of the methyl ester of the acid shows the molecular ion (M^+, 142) and fragments with m/e 127 $(M - CH_3)^+$, 111 $(M - OCH_3)^+$, 99 $(M - 43)^+$, 83 $(M - COOCH_3)^+$, 74 $(CH_3 + COOCH_3)^+$, and 59 $(COOCH_3)^+$. The presence of fairly intense ions with m/e 43 and 99 and the absence of the ion $(M - C_2H_5)^+$ shows that the acid has a branched structure containing an isopropyl group. In the NMR spectrum of the methyl ester, besides the three-proton signals of a methoxy group (6.43 ppm), in the strong field there is a doublet of two methyl groups (8.92 ppm, J = 6.12). This enabled us to identify the acid as 3, 4-dimethylpent-2-enoic acid. This acid, called ikemaic acid [3], was obtained previously in the saponification of cynanchogenin. Its structure was shown by the synthesis of the dihydro derivative [4]. The IR spectrum of the acid that we isolated coincides with that of ikemaic acid (compare Fig. 1 with Fig. 2 in [4]).

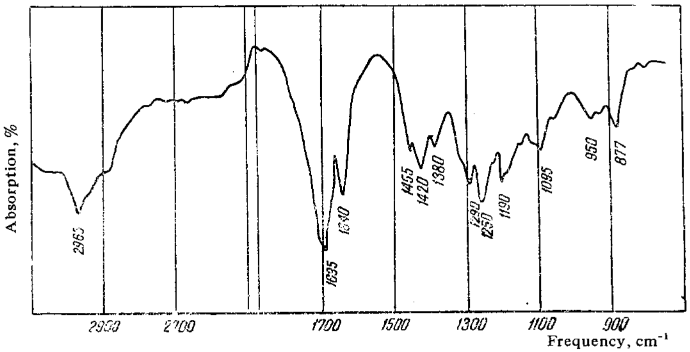


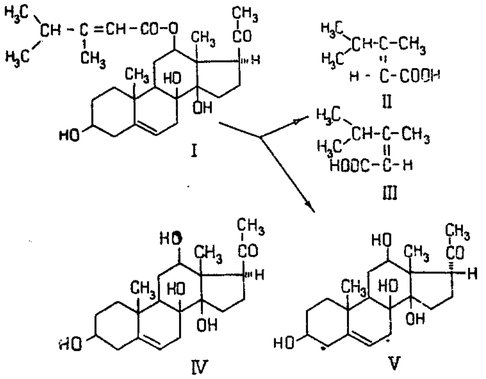
Fig. 1. IR spectrum (liquid film) of 3, 4-dimethylpent-2-enoic acid (III) from sibirigenin (I).

3, 4-Dimethylpent-2-enoic acid is unsaturated, and therefore it should have two geometrical isomers: cis (III) and trans (II). This fact persuaded us to study the spectra in more detail. In its main features, the IR spectrum of the acid differs little from the spectra of α, β-unsaturated acyclic acids; in particular, it is fairly similar to the spectrum of 3-methylnon-2-enoic acid [5]. Disubstituted ethylene derivatives can be ascribed [6] to the trans or cis series fairly accurately by the presence or absence of the nonplanar deformation vibrations of the =CH group at

990–965 cm^{-1}. The situation is different with a trisubstituted double bond. In the acid that we obtained, the nonplanar vibrations of the =CH group appeared clearly at 877 cm^{-1}, but it does not seem possible to ascribe the unsaturated acids to the cis or trans series from this one band [6].

More reliable information was obtained in an analysis of the NMR spectrum of methyl 3, 4-dimethylpent-2-enoate. Study of a large series of cis-trans isomeric pairs of α, β-unsaturated esters shows that carbonyl-containing groups on ethylenic bonds possess well-marked effects of long-range screening with respect to cis β-hydrogen atoms and cis β-methyl groups [7]. It has been found that a trans β-methyl group located in the trans position relative to the ester group is always screened by approximately 0.25 ppm compared with the cis isomer. Consequently, if both isomers of α, β-unsaturated esters are available, this rule can easily be used to determine the configuration of each of them.

We turned our attention to the fact that in those isomers of β-methyl-β-alkyl-substituted esters of unsaturated acids in which the β-methyl group is in the trans position relative to the ester group (dimethyl citraconate, dimethyl cis-β-methylglutaconate, methyl cis-phytenate, methyl cis-α, β-dimethylcinnamate, etc.), the signals of the trans β-methyl protons are not only relatively higher (in the τ scale) by 0.25–0.27 ppm than the signal of the cis β-methyl group, but in absolute magnitude their shifts are greater than 7.95–8.00 ppm [7]. The only exception is methyl trans-geranate. The signals of the $=C-CH_3$ protons of the methyl ester of the acid that we have isolated are found at 7.95 ppm. Consequently, we may assume with a high degree of probability that the natural 3, 4-dimethylpent-2-enoic acid is the cis isomer and corresponds to formula III.



As already stated, the alkaline saponification of sibirigenin gives, besides the acid, a mixture of neutral products. Paper chromatography showed the presence of two components in the mixture. After preparative separation on cardboard, two crystalline compounds were isolated: the more abundant one had mp 246–248° C and the less abundant one mp 250–252° C. Both compounds had the composition $C_{21}H_{32}O_5$, but they differed from one another in their physicochemical constants. The results of a comparison of their properites with those of known pregnane compounds (Table 1) shows that the first of them is lineolon (the difference in the sign of the specific rotation is discussed below), and the second is isolineolon. A direct comparison by chromatography in a thin layer and on paper with authentic samples confirmed the identities of the two pairs of compounds.

Since the individuality of sibirigenin was not a matter of doubt, it could be the ester of lineolon (V) or isolineolon (IV) with ikemaic acid. These two compounds isomerize into one another in alkaline and acid media [3, 9]. If sibirigenin was the ester of lineolon (V), it would be identical with cynanchogenin. A simple comparison of their physicochemical characteristics (Table 2) excluded such a possibility. A direct comparison with authentic cynanchogenin also showed that these compounds were different. The second assumption remains, i. e., that sibirigenin is the ester of isolineolon (IV) and ikemaic acid (III) and has the structure I.

The fact that sibirigenin contains a 17β-oriented side chain is strikingly confirmed by the optical rotatory dispersion curve (Fig. 2). The aglycone shows a positive Cotton effect with a pronounced peak at a long wavelength. The curve of sibirigenin is completely comparable with the analogous curves of 3α-hydroxy-17β-pregnan-20-one [12] and of isolineolon [3]. Conversely, a negative Cotton effect is characteristic for cynanchogenin and lineolon [3]. The optical rotatory dispersion curves of these compounds are similar to that of 3α-hydroxy-17α-pregnan-20-one [12]. Our figure and the figure given in Shimizu and Mitsuhashi's paper [3] both show that the dispersion curve of lineolon does not cross the zero axis anywhere and is completely in the region of negative rotation. The same thing can be said

Table 1

Index	Lineolon = desacylcynanchogenin	Isolineolon = isodesacylcynanchogenin	Products of the alkaline hydrolysis of sibirigenin	
			V	IV
		Aglycone		
Formula	$C_{21}H_{32}O_5$	$C_{21}H_{32}O_5$	$C_{21}H_{32}O_5$	$C_{21}H_{32}O_5$
Mp, °C	238–242 [4,8] 233–239 [8] 242–247 [9]	251–254 [9] 248–249,5 [11]	246–248	250–252
$[\alpha]_D$, deg (in methanol)	+13±2,5 [8,10]	+88,8±2 [9]	–8,4±2 (в этаноле)	+85±2
Color reactions:				
with conc H_2SO_4	Bright red → green [4]	Yellow-brown → green [4]	Bright red → green	Yellow → green
with $SbCl_3$	Violet [9]	Olive [9]	Violet	Olive
UV spectrum: $\lambda_{max}^{C_2H_5OH}$ (log ε)	196 (3,95) 290 (1,97) [8] 195 (4,02) 277 (1,54) [9]	195 (3,97) 277 (1,49) [9]	200 (3,87) 285 (1,83)	200 (3,87) 285 (1,83)
		Diacetate		
Mp, °C	238–241 [4] 243–245 [8] 235–239 [9]	140–141 [9]	233–236	
$[\alpha]_D$, deg (in methanol)	–47,0±2 [8]	+63,8 [9]	–36,5±2	

Table 2

Index	Cynanchogenin [4]	Sibirigenin
Formula	$C_{28}H_{42}O_6$	$C_{28}H_{42}O_6$
Mp, °C	165–167	208–209
$[\alpha]_D$, deg (in ethanol)	–39,5	+54±3
Color reactions:		
Liebermann-Burchard	Pink → yellow	Pink → yellow
with 84% H_2SO_4	Brown → violet	Orange → violet
with $SbCl_3$	Green	Lilac → blue
UV spectrum:		
$\lambda_{max}^{C_2H_5OH}$ (log ε)	218 (4,0)	220 (4,02)
Acetate		
Mp, °C	159	134–135

with respect to the curve of 12-O-benzoyllineolon [13]. Therefore, at the D line (589 mμ) lineolon has a specific rotation with a negative sign. The presence of lineolon in the products of the alkaline isomerization of sibirigenin can be explained by the ease of isomerization of isolineolon.

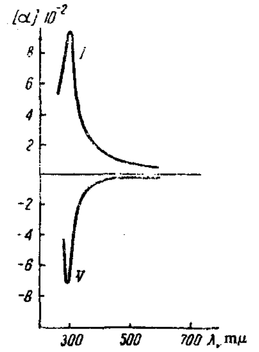


Fig. 2. Optical rotatory dispersion curves of sibirigenin (I) and lineolon (V).

EXPERIMENTAL

The melting points are given without correction for an emergent mercury column. Type KSK silica gel with 5% gypsum was used for thin-layer chromatography. The pregnane aglycones were identified and their purity was checked in a thin layer by the wedged-tip technique [15] in hexane–ethyl acetate (1 : 1) and chloroform–methanol (9 : 1) systems and on paper in a benzene–acetone (2 : 1)/ethylene glycol system. The developing agent was a saturated solution of antimony trichloride in chloroform.

The UV spectra were taken on a Hitachi instrument, the IR spectra in KBr on a UR-10 spectrophotometer, the mass spectra on an MKh-1303 instrument at 110° C with an ionizing voltage of 40 V, and the NMR spectra on a JNM-4H-100/100 MHz instrument with hexamethyldisiloxane (HMDS) as internal standard, the signals of the hydroxyl groups being shifted by means of CF_3—COOH.

The elementary analyses of all the compounds corresponded to the calculated figures.

Isolation of sibirigenin. The ground roots of C. sibiricum were repeatedly extracted with chloroform. The chloroform solution was evaporated to dryness. The residue was dissolved in a small amount of chloroform and the solution was poured into a fivefold volume of petroleum ether. The precipitate was separated off, and reprecipitation was repeated twice. Ten grams of the reprecipitated chloroform extract was dissolved in 50 ml of methanol, and the solution was filtered and mixed with 50 ml of 0.1 N methanolic H_2SO_4 and heated in a water bath for 30 min. Then the solution was cooled, diluted twofold with water, and extracted with chloroform. The chloroform extract was washed with water, dried, and evaporated. The residue was mixed with 2 g of silica gel and transferred to a column containing 300 g of silica gel. Elution was carried out with a mixture of heptane and ethyl acetate with the concentration of the latter being gradually increased from 1 to 50%. The fractions were monitored by thin-layer chromatography.

The heptane–20% ethyl acetate fraction contained a single substance which crystallized after evaporation. Recrystallization from ethyl acetate–heptane gave crystals of sibirigenin in the form of colorless needles (for its properties, see Table 2, and for its optical rotatory dispersion curve, see Fig. 2).

The heptane–30–35% ethyl acetate fraction contained mainly a single compound (thin-layer chromatography). Repeated separation of the fraction on silica gel under the same conditions yielded an amorphous chromatographically individual compound identical with penupogenin $C_{30}H_{40}O_7$ [1, 14].

3-O-Acetylsibirigenin $C_{30}H_{44}O_7$. The substance was obtained by heating a solution of sibirigenin in a mixture of equal amounts of pyridine and acetic acid for 2 hr. Mp 134–135° C (from methanol), $[\alpha]_D^{16}$ +58.8 ± 2° (c 1.64, methanol).

Saponification of sibirigenin (I). A solution of 100 mg of the substance in 5 ml of 5% methanolic caustic potash was boiled for 2 hr. The cooled solution was added to 3 ml of water, and the mixture was extracted with chloroform.

The residue obtained after the distillation of the chloroform was separated on Whatman no. 3 chromatographic paper in a benzene–acetone (2 : 1)/ethylene glycol system. The zones corresponding to lineolon and isolineolon were cut out and separately extracted with ethanol. The ethanolic eluates were evaporated to a small volume, diluted twofold with water, and extracted with chloroform. The residue was recrystallized from ethyl acetate–hexane. The lineolon (V) and isolineolon (IV) obtained in the individual state had the properties shown in Table 1.

The mass spectra of compounds IV and V were fully comparable with those given in the literature [9].

NMR spectrum of lineolon (in C_5D_5N), τ, ppm: 4.16 (2 OH at C_3 and C_{14}), 4.77 (H at C_6), 4.91 (β-OH at C_3 or C_{12}), 5.41 (β-OH at C_3 or C_{12}), 6.24 (2H at C_3 and C_{12}), 6.75 (H at C_{17}), 7.74 (3H at C_{21}), 8.21 (3H at C_{18}), and 8.72 (3H at C_{19}). The optical rotatory dispersion curve is shown in Fig. 2.

3, 12-Di-O-acetyllineolon $C_{25}H_{36}O_7$. The acetate was obtained in the usual way using acetic anhydride in pyridine (for properties, see Table 1). NMR spectrum (in C_5D_5N), τ, ppm: 4.65 (OH at C_8 or C_{14}), 4.85 (H at C_6), 5.12 (2H at C_3 and C_{12}), 6.61 (H at C_{17}), 7.89 (3H at C_{21}), 8.11 (6H, 2Ac), 8.27 (3H at C_{18}), and 8.83 (3H at C_{19}).

Lineolon oxime $C_{21}H_{33}O_5N$. A solution of 20 mg of the substance in 1 ml of methanol was mixed with a solution of 50 mg of hydroxylamine hydrochloride and 65 mg of sodium acetate in 0.5 ml of water. The mixture was boiled in a water bath for 3 hr. On standing, crystals deposited in the form of yellow needles with decomp. p. 260° C (from aqueous methanol).

Interconversions of lineolon (V) and isolineolon (IV). The lineolon from sibirigenin, 5 mg, was boiled in 0.5 ml of 5% methanolic caustic potash for 1 hr. Paper chromatography showed a small spot of isolineolon in addition to lineolon.

Isolineolon (5 mg) obtained from sibirigenin was isomerized as described above. On paper chromatography, the main spot was due to lineolon and a small spot to isolineolon.

cis-3, 4-Dimethylpent-2-enoic (ikemaic) acid (III) $C_7H_{12}O_2$. The aqueous solution remaining after the chloroform separation of the mixture of lineolon and isolineolon (see under the saponification of sibirigenin) was acidified with phosphoric acid and extracted with ether. After evaporation of the ether, a viscous oily liquid remained.

For the IR spectrum, see Fig. 1.

Methyl cis-3, 4-dimethylpent-2-enoate $C_8H_{14}O_2$. The acid III was dissolved in an ethereal solution of diazomethane and left for a day. Evaporation of the solvent yielded a mobile liquid.

UV spectrum: $\lambda_{\max}^{C_2H_5OH}$ 219, 300 mμ (log ε 3.84, 3.03). IR spectrum (liquid film): 1725 (CO), 1645 (C=C), 1235 and 1175 (C—O—C), 870 (=CH).

Mass spectrum, 80° C, m/e (%): 142 (22), 127 (27), 125 (22), 121 (30), 115 (15), 111 (49), 105 (29), 99 (28), 83 (47), 74 (77), 71 (40), 69 (38), 59 (100), 58 (68), 57 (28), 55 (50), 45 (54), 43 (62), and 41 (68).

NMR spectrum (in CCl_4), τ, ppm: 4.47 (H at C_2, doublet, J = 1.5), 6.43 (3H in OCH_3), 7.95 (3H in $=C—CH_3$, singlet), 8.92 (6H in $CH_3—CH—CH_3$, doublet, J = 6.12).

The samples of penupogenin, cynanchogenin, and sarcostin were kindly given to us by Dr. H. Mitsuhashi (Hokkaido, Japan), and the samples of lineolon and 17-isolineolon by Dr. T. Reichstein (Basel, Switzerland), for which the authors express their thanks to them.

The optical rotatory dispersion spectra were taken by I. P. Kovalev (KhNIKhFI [Khar'kov Chemical and Pharmaceutical Scientific-Research Institute]), and the NMR spectra by M. R. Yagudaev.

CONCLUSIONS

The pregnane aglycone sibirigenin from <u>Cynanchum sibiricum</u> has the structure of 12-O-(cis-3, 4-dimethylpent-

2-enoyl)-17-isolineolon.

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9 December 1969

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