SUMMARY

A new direction of the reaction of pennogenin diacetate with $BF_3 \cdot Et_2O$ has been found during which the opening of the spiroketal system is not accompanied by the splitting out of the hydroxy group at C^{17}. It has been shown that one of the products formed in this reaction is 22-oxo-(20S,25R)-cholest-5-ene-3β,16α,17α,26-tetraol 3,16-diacetate. Its fragmentation under electron impact has been studied.

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CARDIAC GLYCOSIDES OF *Acokanthera venenata*

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Six cardenolides have been isolated from the leaves of *Acokanthera venenata* G. Don: AV-1, mp 252-255°C, $[\alpha]_D^{20}$ + 39.4° (MeOH); AV-2, mp 199-208°C, $[\alpha]_D^{20}$ − 59.3° (MeOH); AV-3, mp 269-275°C/300-304°C, $[\alpha]_D^{21}$ − 69.8° (MeOH); AV-4, mp 279-289°C; AV-5, mp 222-225°C, $[\alpha]_D^{20}$ − 64.3° (MeOH); and AV-6, mp 193-196°C $[\alpha]_D^{20}$ − 23.8° (MeOH − $CHCl_3$). AV-5 has been identified as acovenoside A. AV-3 is a new cardiac glycoside: it is 1β-acetoxy-3β-(4'-O-β-D-glucosyl-3'-O-methyl-α-L-talomethylosyloxy)-14-hydroxy-5β,14β-card-20(22)-enolide (gluco-acovenoside B).

Acokanthera venenata G. Don (bushman's poison) is a South African plant containing cardiac glycosides of the cardenolide series. Its seeds which were studied in fairly great detail by Reichstein et al. [1, 2], contain acovenoside A, acovenoside B, acovenoside C, acolongifloroside K, ouabain, and a number of glycosides of unestablished structure [1].

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We have investigated the leaves of bushman's poison grown in the botanical garden of the All-Union Scientific-Research Institute of Medicinal Plants. We have found no information in the literature on the glycoside composition of the leaves of this plant.

From an alcoholic extract we isolated the combined cardiac glycosides with a yield of 2.2% on the weight of the leaves and with high degree of purity. Analysis with respect to one of the main cardiac glycosides (AV-3, see below) indicated that the amount of glycosides in the extract was more than 90%.

Chromatographic analysis and the use of specific reagents showed that the glycosides of bushman's poison leaves belonged to the cardenolide group and their total number was not less than eight.

Six cardenolides were isolated by adsorption chromatography on silica gel, and these have been provisionally designated as AV-1—AV-6. The main ones in relative amount were AV-1 and AV-3.

The properties of AV-5 corresponded to the known glycoside acovenoside A [3], which is 1β,14-dihydroxy-3β-(3'-O-methyl-α-L-talomethyloxyloxy)-5β,14β-card-20(22)-enolide. The acid hydrolsis of AV-1 gave an aglycon identical with a sample of acovenosigenin A.

AV-3 was a new cardiac glycoside, with the composition $C_{38}H_{58}O_{15}$. On enzymatic hydrolysis with the pancreatec juice of the grape snail, acovenoside A and D-glucose were formed. The IR spectrum of AV-3 contained adsorption bands with maxima at 3170 and 1243 cm^{-1} relating to an acyl group. The PMR spectrum characterized this group as an acetyl group — a three-proton singlet with a chemical shift δ = 2.2 ppm.

The peak of the molecular ion was absent from the mass spectrum of AV-3. An ion with m/z 433 and the composition $C_{25}H_{37}O_6$ was the ion of the protonated aglycon — acetylacovenosigenin A. The splitting out of the substituents from this fragment gave ions with m/z 415, 414, 397, 372, 355, 354, 337, 336, and 321. The key ions of the cardenolide series were represented by fragments with m/z 219, 201 (a), 262, and 244 (c) [3], which corresponds to the presence of two oxygen substituents in rings A-C.

The acetoxy group in the aglycon of acovenosigenin can feasibly be located only at C-1. An important confirmation of the presence of a 1β-OAc group was provided by information on the biological activity of AV-3, which showed that this glycoside had a low activity — animals given the substance in a dose of 3 mg/kg did not die. It is known that 1β-O-acetyl-glycosides exhibit a low biological activity in the Hatcher test. Thus, acovenoside B, having such a grouping, exhibits a LD_{100} of 2.14 mg/kg weight of a cat.

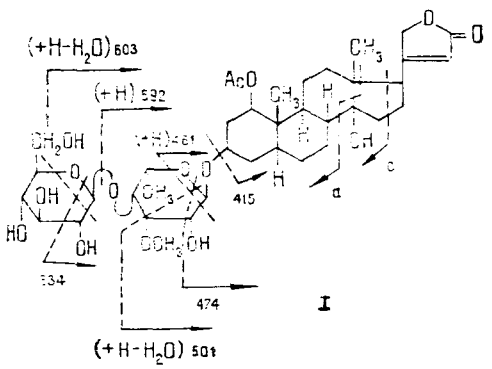
The butenolide ring in AV-3 has the β-configuration, as is shown by the formation from it of the known aglycon acovenosigenin A, and also by the presence in the PMR spectrum of a signal in the form of a quartet with δ = 2.81 ppm relating to the 17α-H proton [4, 5].

Of the two possible positions of the attachment of the D-glucose residue to the 3-O-methyl-L-talomethylose unit — C-2' and C-4' — the choice was made in favor of C-4' on the basis of the fact that in AV-3 the D-glucose unit is hydrolyzed by enzyme preparations, while analogous glycosides in which a D-glucose residue is attached at C-2' are scarcely hydrolyzed under these conditions.

The 3-O-methyl-L-talomethylose residue in AV-3 is attached by an α-glycosidic bond, as is shown by the molecular rotation increment ($\Delta C = [M]_{D,AV-3} - [M]_{D,acovenosigenin\ A} = -350°$) and the PMR spectrum. In the PMR spectrum the anomeric proton of this monosaccharide unit gives a signal in the 5.09 ppm region with the SSCC J = 1.2 Hz. In view of the fact that L-talomethylose in glycosides preferentially has the $^1C_4$-configuration [6], this indicates the equatorial orientation of the anomeric proton. The D-glucose residue is attached by a β-glycosidic bond ($\Delta C_{D\text{-glucose}} = [M]_{D,AV-3} - [M]_{D,AV-5} = -170°$). The anomeric proton of this unit is axial — in the PMR spectrum it appeared in the form of a doublet at δ = 4.58 ppm, J = 9 Hz.

Thus, AV-5 can be characterized as 1β-acetoxy-3β(4'-O-β-D-glucosyl-3'-O-methyl-α-L-talomethylosyloxy)-14-hydroxy-5β,14β-card-20(22)-enolide (I). An abbreviated (semitrivial) name for this glycoside may be "glucoacovenoside B" (see following page).

The presence of carbohydrate units is characterized in the mass spectrum of (I) by the following fragments. The ion with the greater mass, m/z 634, corresponds to the breakdown of the glycopyanose unit at the C_1—O and C_2—C_3 bonds (see formula I). Another pathway of the



breakdown of the terminal hexose gives an ion with m/z 603. An ion with m/z 592 practically coincides with the M^+ ion of 1-acovenoside A. In the spectrum of (i) all three types of breakdown of the talomethylose unit characteristic of cardenolide glycosides and other classes of natural compounds were observed [7]. The origins of all the fragments were confirmed by measurements of elementary compositions.

EXPERIMENTAL

The IR spectrum was taken on a IR-27G spectrometer, the PMR spectra on a Bruker WM-250 spectrometer at 400 MHz in C_5D_5N (0 — TMS), and the mass spectrum and the compositions of the ions were obtained on a MKh 1310 instrument. Elementary analysis was performed with the aid of an automatic C—H—N analyzer, and the results of the analyses of the compounds corresponded to the calculated figures. Melting points were determined on a Kofler stage.

The absorption-chromatographic separation and the purity of the substances obtained were checked by paper chromatography using the solvent systems methyl ethyl ketone—m-xylene (1:1) formamide, chloroform—tetrahydrofural (1:1)/formamide, and benzene/formamide.

Isolation of the Glycosides. Dry leaves of bushman's poison grown and collected in 1984 (200 g) were extracted exhaustively with 96% ethanol. The solution was concentrated to a volume of 0.2 liter, diluted with 0.4 liter of water, and filtered through 100 g of alumina. The adsorbent was washed with 35% ethanol until the reaction for cardenolides was negative. The glycosides were extracted from the filtrase first with chloroform (2 ××2 liter) and then with a mixture of chloroform and ethanol (2:1; 4 × 1 liter). The combined chloroform and ethanol-chloroform extracts were evaporated in vacuum. The residue (5 g) was chromatographed on 0.9 kg of silica gel, using mixtures of chloroform and methanol of increasing polarity, beginning with a ratio of 99:1, as eluent. The cardenolides were crystallized from methanol. In this way, the following compounds were obtained:

AV-1, mp 252—255°, $[\alpha]_D^{20} + 39{,}4 \pm 3°$ (*c* 1,0; MeOH);

AV-2, mp 199—203°, $[\alpha]_D^{20} - 59{,}3 \pm 3°$ (*c* 1,0; MeOH);

AV-3, mp 269—275°/300—304°, $[\alpha]_D^{21} - 69{,}8 \pm 3°$ (*c* 1,0; MeOH);

AV-4, mp 279—289°;

AV-5 (acovenoside A), 222—225°; $[\alpha]_D^{20} - 64{,}3 \pm 3°$ (*c* 1,0; MeOH);

AV-6, mp 193—196°; $[\alpha]_D^{20} - 23{,}8 \pm 4°$ (*c* 1,0; MeOH—$CHCl_3$).

Mass spectrum of (I), m/z (%): 634 (0.6), 606 (0.8), 603 (0.5), 592 (0.9), 550 (592 — CH_2CO; 12.5), 548 (M — $4H_2O$ — CH_2O — AcOH — CO_2; 0.65), 514 (592 — AcOH — H_2O), 501 (0.8), 474 (1.1), 461 (4.0), 443 (2.5), 433 (7.5), 432 (3.0), 415 (23), 414 (15), 401 (5.0), 397 (11), 372 (37), 355 (74), 354 (100), 337 (61), 336 (35), 321 (8.0), 262 (7.5), 244 (17), 219 (17), 201 (96).

Enzymatic Hydrolysis of AV-3. A solution of 50 mg of the glycoside in 0.5 ml of dimethylformamide and a separately prepared aqueous solution of the enzyme preparation obtained from the pancreatic juice of the grape snail (150 mg in 10 ml) were mixed and the mixture was left at 42°C for seven days, the process of hydrolysis being monitored with the aid of paper chromatography. After the end of the reaction, the mixture was diluted with 40 ml ethanol, the mixture was heated to the boil, and the precipitate of enzymes was separated off.

A monoglycoside was extracted from the filtrate with chloroform (4 × 80 ml) and the extract was filtered through a layer of alumina (1 g, activity grade III) and was evaporated. The residue was crystallized from ethanol. The properties of the monoglycoside [mp 221-225°C; $[\alpha]_D^{20}$ − 64.5 ± 3° (s 0.8; MeOH)], corresponded to those of acovenoside A.

The aqueous phase was evaporated to dryness. According to paper chromatography, the residue contained D-glucose. The phenylosazaone of this monosaccharide was obtained, with mp 207-208°C; it was identical with the phenylosazone of D-glucose.

SUMMARY

Six cardenolides have been isolated in the individual state from the leaves of *Acokanthera venenata* G. Don. They include acovenoside A and a new cardiac glycoside which has been named glycoacovenoside B and has been characterized from the results of the investigation as 1β-O-acetyl-3β-(4'-O-β-glucopyranosyl-3'-O-methyl-α-L-talomethylsyloxy)-5β,14β-card-20(22)-enolide.

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TRITERPENE GLYCOSIDES OF *Thalictrum squarrosum*

I. STRUCTURE OF SQUARROFURIC ACID

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Squarrofuric acid has been isolated from *Thalictrum squarrosum* by the acid hydryolysis of a methanolic extract. It is suggested that is is an artefact formed on hydrolysis and has the structure of 3β,30-dihydroxy-22,25-epoxylanost-9(11)-en-21-oic acid. ^1H and ^{13}C NMR spectra are given for squarrofuric acid and nine of its derivatives. The mass-spectral characteristics and physicochemical constants of the compounds studied are presented.

In a study of the saponins of plants of the genus *Thalictrum* [1, 2] we found that *Thalictrum squarrosum* Steph. (nodding meadow rue) contains not less than eight triterpene glycosides (about 1% of the weight of the raw materials). We have called the saponins of this species squarrosides. Some sapogenins of these glycosides belong to the tetracyclic triterpenoids with a cyclopropane ring.

The acid hydrolysis of a metabolic extract of nodding meadow rue gave two predominating sapogenins. In the present communication we consider the structure of the main product of the hydrolysis of the glycosides — genin I, which we have called squarrofuric acid (Scheme on following page).

Squarrofuric acid (I) — $C_{30}H_{48}O_5$, M^+ 488 — is a triterpene acid (IR: 1675 cm^{-1}; ^{13}C NMR: 175.8 ppm) having, according to its PMR spectrum, six methyl groups (0.74, 0.92, 0.97,

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