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NEW CARDENOLIDES FROM THE LEAVES OF *Gomphocarpus fruticosus*

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Two new glycosides, gomphotin (1) and gomphotoxin (13), and also the known gomphoside (9) have been isolated from the leaves of Gomphocarpus fruticosus. *Compounds (1) and (9) contain a 4,6-dideoxyhexosulose residue as the sugar component, and (13) a 6-deoxyhexosulose, which are attached to glycolic OH groups of the aglycons by 3β−O−1'α,2α−O−2'α (9, 13) and 3β−O−1'α,4β−O−2'β (1) acetal-ketal bonds. The structures of the new compounds are represented by the names (3β−O−1'α,4β−O−2'β)-(2'α,3'α-dihydroxy-4',6'dideoxyhexulosido)-14β-hydroxy-5α-card-20(22)-enolide* (1) and (3β−O−1'α,2α−O−2'α)-(2'β,3'α,4'β-trihydroxy-6'-deoxyhexulosido)-14β-hydroxy-5α-card-20(22)-enolide* (13).*

The isolation from the leaves of *Gomphocarpus fruticosus* (L.) R.Br. of six substances of cardenolide nature, of which uzarigenin and deglucouzarin were identified, has been reported previously [1]. In the present paper we give the results of a determination of the structures of two new glycosides, which have been called gomphotin (**1**, substance **3**) and gomphotoxin (**13**, substance **5**) and also of the identification of gomphoside (**9**), which has been isolated previously from the same plant species by Australian researchers [2].

The substances isolated (**1**, **9**, **13**) gave a positive reaction for 6-deoxyhexosones with dinitrophenylhydrazine [3, 4]. On high-vacuum thermal decomposition they formed methylreductic acid (**4**). Their UV spectra showed a single absorption maximum in the 220 nm region (log ε 4.35-4.40), which is typical for the butenolide lactone rings of cardenolides, the presence of which was confirmed by IR spectra (absorption bands at 1633 and 1750 cm^{-1}).

The positive course of the curves in the optical rotatory dispersion (ORD) spectra (Fig. 1) and absorption bands in the 1230-1240 cm^{-1} region showed the *trans* linkage of the *A/B* rings in the steroid nuclei and the absence of double bonds at C-5 and C-14 [6]. The acid hydrolysis of (**1**, **9**, and **13**) formed the 14-anhydroaglycons (**5**) and (**11**), the positions of the double bonds in which were confirmed by treating their acetates (**7** and **12**) with thionyl chloride in pyridine [5]. The ORD spectra (Fig. 1) of the 14-anhydroaglycons of glycosides (**9**) and (**13**) each had a negative course and an intense absorption band in the 851 cm^{-1} region. Both the initial glycosides (**1**, **9**, **13**) and their anhydroaglycons reacted with $NaIO_4$ and gave a positive benzidine−periodate test on silica gel plates [4] showing the presence of α-glycol groupings in their molecules. [see scheme on following page].

Gomphotin (1). Together with the above-mentioned common properties for the substances under investigation, gomphotin formed a diacetyl derivative (**2**) and an acetonide (**3**), which shows the *cis*-orientation of the hydroxyls in the glycol grouping of the 4',6'-dideoxyhexazone moiety. Such a configuration of the hydroxyls is possible if one of the semiketal OH groups at C-2' of the 4',6'-dideoxyhexazone is bound with the aglycon by an equatorial 2'β−O−4β-ketal bond and the second OH group remains free and occupies the axial (α) position (**1**). As in gomphoside, the hydroxyl at C-3' occupies the equatorial position (**9**).

*The original nomenclature, with minor modifications, is used throughout. A more rational nomenclature is exemplified by the Chemical Abstracts name for gomphoside (**9**): [2α(2S,3S,4R,6R,3β,5α]-14-hydroxy-2,3-[tetrahydro-3,4-dihydroxy-6-methyl-2*H*-pyran-2,3-diyl)bis(oxy)]card-20(22)-enolide.

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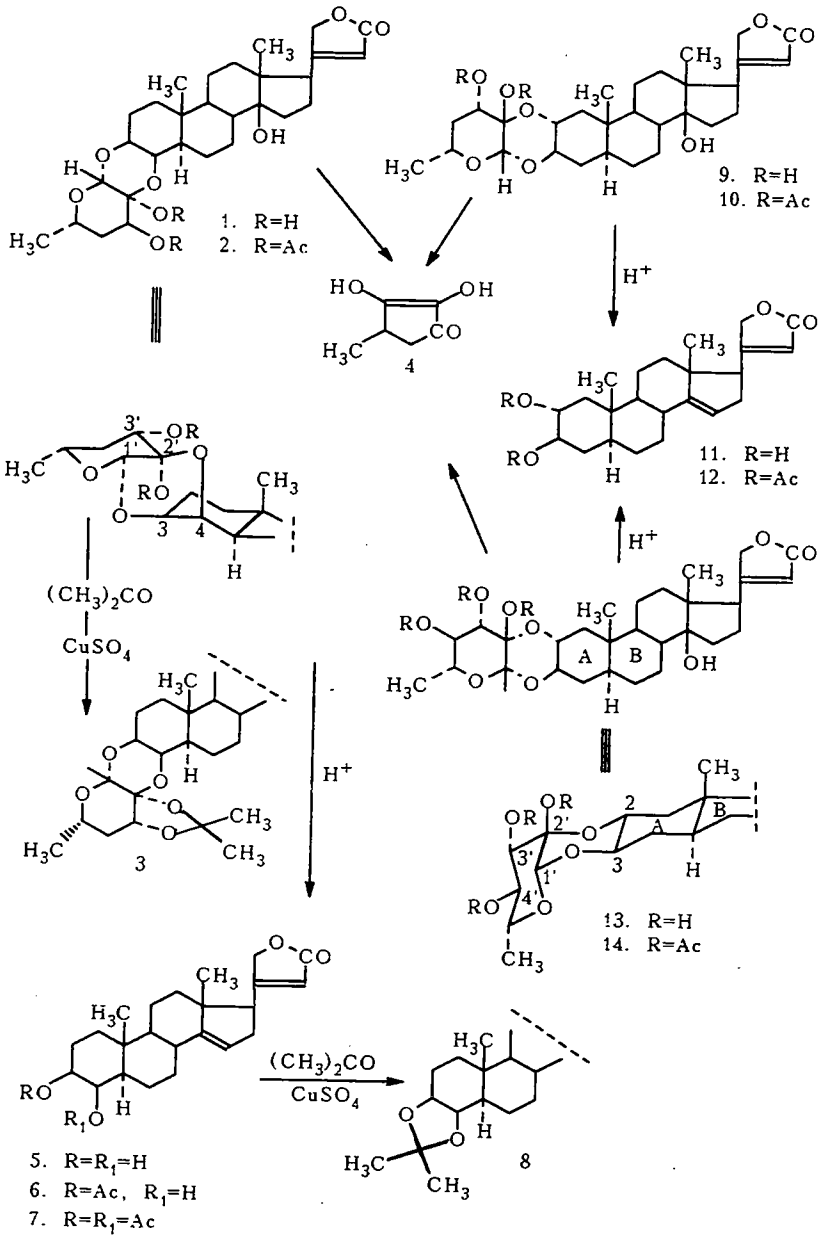

The acid hydrolysis of gomphotin (**1**) gave 14-anhydrogomphotinogenin (**5**), which, depending on the acetylation conditions, formed a monacetate (**6**) or a diacetate (**7**) because of the axial position of one of the OH groups [7]. An IR absorption band in the 1240 cm^{-1} region showed the equatorial (β) position of the acetoxy group at C-3 of the steroid skeleton of (**5**) and the *trans*-linkage of the *A/B* rings [3], as was confirmed by the high biological activity of gomphotin (0.13-0.18 mg/kg body weight of a cat). As is known, cardenolides with the α-orientation of the OH group at C-3 have a low biological activity [9]. The capacity of 14-anhydrogomphotinogenin (**5**), in contrast to gomphogenin (**11**) [2, 4], for forming an acetonide (**8**) showed the presence of a *cis*-glycol grouping, with the most probable position of the hydroxyls at 2β(a)-3β(e) or 3β(e)-4β(a), since OH groups in the 2α(e)-3β(e) and 3β(e)-4α(e) positions, being in the *trans* position to one another, do not form an acetonide. A comparison of the molecular rotation increments of acetyl groups present in the 2α, 2β, 4α, and 4β position in nuclei of the *trans*-A/B series [4, 11] showed that the second acetoxy group in the aglycon (**7**) under investigation is present at C-4 and has the β- (a-) orientation (Table 1), confirmation of this being the low rate of acetylation of the OH group in this position [7].

On the basis of the results obtained, the structure of gomphotinogenin can be represented as 3β,4β,14β-trihydroxy-5α-card-10(22)-enolide, and its glycoside gomphotin as (3β−O−1′α,4β−O−2′β)-(2′α,3′α-dihydroxy-4′,6′dideoxyhexulosido)-14β-hydroxy-5α-card-20(22)-enolide.

TABLE 1. Determination of the Positions and Configurations of the Acetyl Groups in 14-Anhydrogomphotinogenin Diacetate (7) by a Comparison of Molecular Rotations

Substance	$[\alpha]_D$*	$[M]_D$	$\Delta[M]_D$	Position and configuration
	degrees			
Δ^{14}-Gomphotinogenin diacetate	-33.0	-150.7		
Δ^{14}-Uzarigenin 3-monoacetate [4]	-27.0	-107.2		
Proportion of the molecular rotation of an acetoxy group in Δ^{14}-gomphotinogenin diacetate			-43.2	4β
Δ^{14}-Gomphogenin diacetate (12)	-67.5	-308.3		
Δ^{14}-Uzarigenin 3-monoacetate [4]	-27.0	-107.2		
Proportion of the molecular rotation of an acetoxy group in Δ^{14}-gomphogenin diacetate			-201.1	2α
Proportions of the molecular rotation of the acetyl residues at C-2 and C-4 of diacetoxycholestane [4]			-188.0	2α
			+129.1	2β
			+27.5	4α
			-89.9	4β

*In chloroform.

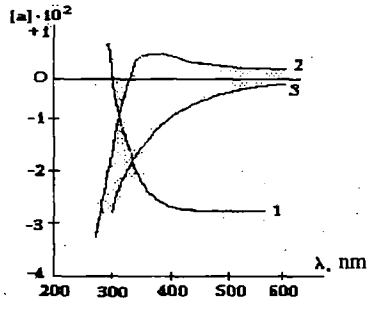

Fig. 1. Optical rotatory dispersion spectra (in MeOH): 1) gomphotin (**1**); 2) 14-anhydrogomphotinogenin (**5**); 3) 14-anhydrogomphogenin (**11**).

Gomphoside (9). Substance (**9**) had the same molecular mass and empirical composition as gomphotin and a number of the other properties discussed above. Neither glycoside (**9**) nor 14-anhydrogomphogenin formed an acetonide, in spite of the presence of glycol groupings in their molecules [2, 4]. On the basis of the physicochemical properties of glycoside (**9**) and the products of its transformation, and also a direct comparison with an authentic sample,* the substance under investigation was identified as gomphoside [2, 4]: (3β−O−1α,2α−O−2′α)-(2′β,3′α-dihydroxy-4′,6′-dideoxyhexulosido)-14-hydroxy-5α-card-20(22)-enolide.

Gomphotoxin (13). On acetylation, substance (**13**), with the empirical formula $C_{29}H_{44}O_9$ gave a triacetyl derivative (**14**). The sugar component in (**13**) is a hexosone [4]. This glycoside did not form an acetonide, From the products of the acid hydrolysis of (**13**) we isolated a 14-anhydroaglycon (**11**), which was identified from its physical properties, R_f values in a series of solvents, and IR spectra as 14-anhydrogomphogenin.

In view of the structure of the aglycon (**11**), it can be stated that all three hydroxyls capable of being acetylated are present in the hexosuloside part of the molecule of the substance concerned (**13**). The resistance of (**13**) to the formation of an acetonide gives grounds for concluding that the OH groups are in the *trans* position to one another. And if we take as a basis the assumption that the most stable conformation of the hexosulose moiety is a "chair" with equatorial groups at C-2′ and C-3′, as has been proposed for gomphoside [4], the hydroxyl at C-4′ will also be equatorial (**13**). If even one OH group had the axial orientation, as in gomphotin (**1**), the formation of an acetonide would proceed readily.

*The authors express their gratitude to Professor T. R. Watson (Sidney University, Australia) for providing the sample of gomphoside.

TABLE 2. Determination of the Configurations of the OH Groups at C-4′ in the 6-Deoxyhexosulose Moieties of the Molecules of Gomphotoxin (13) and Calotoxin by a Comparison of Molecular Rotations

Substance	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$	Configuration at C-4′
	degrees			
Gomphotoxin, (13) ($C_{29}H_{42}O_9$)	+23.5	+125.5		
Gomphoside (9) ($C_{29}H_{42}O_8$)	+14.4	+74.4		
Proportion of the molecular rotation at C-4 of gomphotoxin			+50.8	β
Calotoxin ($C_{29}H_{40}O_{10}$) [4]	+66.0	+362.1		
Calactin ($C_{29}H_{40}O_9$) [4]	+57.3	+305.2		
Proportion of the molecular rotation of the OH group at C-4′ of calotoxin			+56.9	β

Thus, gomphotoxin is 3β−O−1′α,2α−O−2′α-(2′β,3′α,4′β-trihydroxy-6′-deoxyhexulosido)-14β-hydroxy-5α-card-20(22)-enolide (**13**). As can be seen, (**13**) differs from gomphoside (**9**) only by an additional OH group in the 4′β(e) position. A similar pair with respect to the number of hydroxy groups in the hexosuloside moieties is kalotoxin and kalaktin [4], the aglycon of which is 2α,3β,14-trihydroxy-19-oxo-5α-card-20(22)-enolide. In our opinion, the conformations of the nuclei of the glycoside molecules and of the hydroxyls in these two pairs of cardenolides should be the same, and this has been confirmed by the method of molecular rotation differences [10, 11] Table 2). As can be seen from Table 2, $\Delta[M]_D$ for the 4′β-OH group of gomphotoxin (**13**) and the increment for the hydroxyl at C-4′ of calotoxin have the same sign and similar absolute magnitudes, which indicates an identity of the structures of the 6′-deoxyhexosulose moieties and their conformations in gomphotoxin and gomphoside and in calotoxin and calactin [4, 14a-e]. It must be mentioned that hitherto only a very small number of glycosides with acetal (3−O−1′)-ketal-(2−O−2′) or -(4−O−2′) bonds between the carbohydrate moiety and the aglycon have been isolated.

EXPERIMENTAL

Gomphotin (1) (substance 3) and **gomphotoxin (13)** (substance 5) were isolated from the fermented and unfermented raw material.

Adsorption chromatography was conducted on neutral alumina. For analysis the substances were dried in vacuum (10^{-2} mm Hg) at 110-115°C for 4-5 h over P_2O_5; melting points were determined on a Kofler block; UV spectra were taken on a SF-26 spectrophotometer and IR spectra on a UR-20 instrument (tablets with KBr); and optical rotations were determined on a ÉPL-IA instrument. Optical rotatory dispersion spectra were obtained on a SPU-M instrument. Temperatures are given in degrees Celsius.

Isolation of Gomphoside (9) from *G. fruticosus* Leaves. After the isolation of gomphotin (1), the mother liquors were crystallized, and the crystals that had deposited were filtered off and dried. The paper chromatography of these crystals in the solvent systems chloroform−formamide and benzene−chloroform (1:3):formamide systems gave a single spot at the level of gomphotin (1). A solution of 300 mg of the crystals in 250 ml of anhydrous acetone was treated with 15 g of finely ground anhydrous $CuSO_4$, and the mixture was boiled in a flask with a reflux condenser and a calcium chloride tube for 6 h. After the elimination of acetone from the reaction mixture, the acetonide formed was separated in a thin layer of alumina, which was washed with benzene. The eluate was evaporated, and the residue was crystallized from methanol giving 107 mg of the acetonide (**3**). The substance (**9**) that had not reacted was eluted from the column with chloroform and, after evaporation of the solvent, was crystallized from alcohol (139 mg).

Gomphotin (1). The substance crystallized from aqueous methanol in the form of thin rectangular crystals with mp 227-230°C, $[\alpha]_D^{20}$ + 39 ± 2° (*c* 0.9; MeOH). Found, %: C 67.04, H 8.28; $C_{29}H_{42}O_8$ (518). Calculation, %: C 67.2, H 8.30.

UV spectrum (nm): 220 (log ε 4.50). Optical rotatory dispersion (ORD) spectrum: Fig. 1, curve *1*. IR spectrum (ν, KBr, cm^{-1}): 3320, 2880, 2815, 2800, 1740, 1685, 1550, 1400, 1310, 1240.

On paper chromatography in a chloroform−formamide system. gomphotin had R_f 0.64.

Gomphotin Diacetate (2). A solution of 200 mg of (**1**) in 4 ml of anhydrous pyridine was treated with 4 ml of acetic anhydride, and the reaction mixture was left for two days and was then heated at 45-50°C for 30 min. After cooling, it was

poured into 50 ml of ice water. The precipitate that deposited was filtered off and recrystallized from ethanol. The resulting acetyl derivative (2) (178 mg) melted at 257-262°C, $[\alpha]_D^{20}$ + 22 ± 2° (*c* 0.92; MeOH).

Found, %: C 65.31, H 7.92; $C_{33}H_{46}O_{10}$ (602.3). Calculation, %: C 65.8, H 7.63.

Reaction product (2) was found to contain two acetyl residues [12].

The Benzidine–Periodate Reaction. To perform the test for the presence of α-glycol groupings in (1), (5), (9), (11), and (13), a silica gel plate was prepared upon which solutions of the substances under investigation were deposited and were treated with the reagents described below. A positive reaction was characterized by a white spot on a dark background.

Preparation of a SiO_2 Plate. A suspension of 5 g of type KSK silica gel with a particle size of 0.25 mm and 0.3 g of gypsum in 5 ml of water and 5 ml of ethanol was deposited on a small glass plate in an amount calculated to give a layer thickness of about 0.2 mm [sic]. The plate was dried in the air for 30-40 min and then in a drying chest at 150°C for 30 min.

Reagent 1. A solution of 6.4 g (0.03 mole) of $NaIO_4$ in 750 ml of water to which 250 ml of *tert*-butanol had been added.

Reagent 2. a) A solution of 5.5 g (0.03 mole) of benzidine in 500 ml of *tert*-butanol; b) a solution of 48.0 g (0.6 mole) of NH_4NO_2 in 500 ml of water.

To perform the reaction for an α-glycol grouping we took about 0.1-0.15 mg of substance, dissolved it in the minimum amount of methanol, and deposited the solution on the prepared silica gel plate. It was necessary that the diameter of the deposited spot should not exceed 4 mm. After the drying in the air of the solution of the substance under investigation deposited on the plate, it was sprayed with reagent (1) and left in the air for 5 min. Then the plate was placed for 1 h in a chamber saturated with a mixture of water and *tert*-butanol in a ratio of 3:1. After the lapse of the indicated time, the plate was removed and was sprayed copiously with a freshly prepared mixture of reagents a and b. At the position of deposition of substance (1) a white spot appeared on a dark background, showing the presence of an α-glycol grouping in it. The benzidine–periodate reaction was also positive for substances (5), (9), (11) and (13).

The Acetonide (3) from (1). Acetonide (3) was obtained during the separation of substance (1) from (9) (see above); mp 241-245°C (from aqueous alcohol). Found, %: C 68.65, H 12.31; $C_{32}H_{46}O_8$ (558). Calculation, %: C 68.83, H 8.23.

When the isopropylidene residue was split out, the initial substance (1) was obtained.

Acid Hydrolysis of (1). A solution of 500 mg of (1) in 25 ml of methanol was treated with 25 ml of 8% H_2SO_4, and the reaction mixture was heated in the boiling water bath for 2 h. When the hydrolyzed mixture was subjected to paper chromatography in the chloroform–formamide solvent system, no (1) was detected. The hydrolysate was evaporated to 25 ml and left in the cold for a day. The crystals that deposited were filtered off, washed with water, and recrystallized twice from methanol. This gave 210 mg of colorless crystals with mp 193-197°C, $[\alpha]_D^{20}$ + 22 ± 3° (*c* 0.2; MeOH).

Found, %: C 73.96, H 8.71; $C_{23}H_{32}O_4$ (372.48). Calculation, %: C 74.75, H 8.75.

As a result of the acid hydrolysis of (1), the OH group at C-14 in the aglycon was eliminated, as was confirmed by treating the aglycon diacetate (7) that had been obtained with thionyl chloride in pyridine [4, 13]. This re-formed the initial substance (5) (14-anhydrogomphotinogenin).

14-Anhydrogomphotinogenin (5) gave a positive benzidine–periodate reaction, showing the presence of an α-glycol grouping in it (for the performance of the reaction, see above).

Methylreductic Acid (4) from (1). A small retort with a side-tube having globular widenings was charged with 600 mg of gomphotin (1) and was heated in an oil bath to 240-245°C under a vacuum of 0.01-0.02 mm Hg. After 40-50 min, heating was stopped and the retort was cooled. This gave 110 mg of colorless acicular crystals with mp 83-84°C and the empirical formula $C_6H_8O_3$. The substance (4) obtained as the result of pyrolysis was hygroscopic and readily soluble in water, and its aqueous solution reduced Fehling's solution in the cold, formed a red coloration with the Legal reagent [14], and, with phenylhydrazine, gave an osazone with mp 148-149°C. From its physicochemical properties and its osazone, (4) was identified as methylreductic acid. This was confirmed by the formation of a red-orange precipitate of the dinitroophenylosazone when HCl was added to the reaction mixture, and an alcoholic solution of this was blue or violet-blue [4], which is characteristic for the dinitrophenylosazone of methylreductic acid. Glycosides with normal sugars do not give such a coloration. Methylreductic acid was also obtained from (9) and (13).

3-Mono-O-acetyl-14-anhydrogomphotinogenin (6). A solution of 300 mg of the anhydrogenin (5) in 10 ml of anhydrous pyridine was treated with 10 ml of acetic anhydride, and the mixture was left at room temperature for 15 h. Paper chromatography of the reaction mixture in benzene–formamide showed that the initial substance was absent, while two new derivatives were detected, the main one of which, (6), had R_f 0.6, while there was only a very small amount of the second,

(7), less polar one, with R_f 0.88. Substance (6) was crystallized from hexane–benzene (236 mg): mp 165-170°C, $[\alpha]_D^{20}$ + 12.3 ± 3° (*c* 0.1; chloroform), $C_{25}H_{34}O_5$. Compound (6) was found to contain one acetyl group.

3,5-Di-O-acetyl-14-anhydrogomphotinogenin (7). A solution of 200 mg of the 14-anhydroaglycon (5) in 7 ml of anhydrous pyridine was treated with 7 ml of acetic anhydride, and then the reaction mixture was heated to 40-50°C for 17 h. This gave substance (7) with R_f 0.88, which was crystallized from aqueous alcohol (205 mg): mp 244-252°, $[\alpha]_D^{21}$ — 33 ± 3° (*c* 0.1; chloroform), $C_{27}H_{36}O_6$. Two acetyl groups were detected in substance (7).

The Acetonide of 14-Anhydrogomphotinogenin (8). A solution of 40 mg of anhydrogomphotinogenin (5) in 4 ml of absolute acetone was treated with 200 mg of anhydrous $CuSO_4$, and the reaction mixture was treated further as described above. The reaction product was crystallized from 70% alcohol, giving 39 mg of substance (8) with mp 264-268°C, $C_{26}H_{36}O_4$.

Gomphoside (9). The substance had the empirical formula $C_{29}H_{42}O_8$, mp 235-244°C, $[\alpha]_D$ — 14 ± 2° (*c* 0.8; MeOH), and gave a single absorption maximum in the UV spectrum at about 220 nm (log ε 4.48) On thermal decomposition, (9) formed methylreductic acid (4). It was not reduced by $NaBH_4$ and did not form an acetonide; its benzidine–periodate test was positive.

Gomphoside Diacetate (10). A solution of 200 mg of (9) in 4 ml of pyridine and 4 ml of acetic anhydride was heated to 45-50°C for 30 min, and the acetylation product was isolated as described for (2). This gave 197 mg of crystalline (10) with mp 252-254°C, $[\alpha]_D$ + 32 ± 2° (*c* 1.0; $CHCl_3$), $C_{33}H_{48}O_{10}$.

14-Anhydrogomphogenin (11). Compound (9) (100 mg) was hydrolyzed in the same way as gomphotin (1). Two recrystallizations from aqueous methanol yielded 43 mg of (11) ($C_{23}H_{32}O_4$, mp 230-236°C, $[\alpha]_D$ +6 ± 2° (*c* 1.0; MeOH). The 14-anhydrogomphogenin (11) obtained gave a positive benzidine–periodate test and did not form an acetonide.

14-Anhydrogomphogenin Diacetate (12). The diacetyl derivative of (12) was obtained in a similar way to (10) ($C_{27}H_{36}O_6$, mp 239-246°C $[\alpha]_D^{21}$ — 67.5 ± 2° (*c* 0.5, $CHCl_3$). Two acetyl groups were found in (12).

Gomphotoxin (13). The substance crystallized from aqueous alcohol in the form of needles with mp 241-243°C, $[\alpha]_D^{21}$ +23 ± 2° (*c* 0.8; MeOH). Found, %: C 65.7, H 8.09; $C_{29}H_{42}O_9$ (534.3). Calculation %: C 65.14, H 7.91.

The UV spectrum of (13) had a single absorption maximum in the 220 nm region (log ε 4.35); the ORD spectrum was a smooth positive curve. The substance did not change under the action of $NaBH_4$ and of ammoniacal methanol [1]. Its biological activity was 0.35 mg per 1 kg body weight of a cat.

The Benzidine–Periodate Test. The reaction was carried out by the method described above and proved to be positive for (13).

Formation of an Acetonide. A solution of 20 mg of (13) in 2.5 ml of anhydrous acetone was treated with 100 mg of $CuSO_4$. The reaction mixture was then treated as for substance (1). The starting material was recovered.

Thermal Decomposition of Gomphotoxin (13) to (4). On thermal decomposition performed as for (1), substance (13) formed methylreductic acid (4).

Gomphotoxin Acetate (14). The acetylation, as for (1), of 300 mg of (13) gave 286 mg of the acetyl derivative (14) with mp 203-205°C, $C_{35}H_{49}O_{12}$, in which three acetyl groups were detected.

Hydrolysis of Gomphotoxin (13). The hydrolysis of 250 mg of (13), as for (1), gave 107 mg of a 14-anhydroaglycon identical with the 14-anhydrogomphogenin (11) described above.

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