ALKALOID CARDENOLIDES

I. F. Makarovich, L. V. Ivanov,
Ya. I. Khadzhai, V. F. Belokon',
V. V. Pavlova, O. I. Klimenko,
N. Ya. Bondar', and E. V. Uryupina

UDC 547.92/.93;547.94

New alkaloid cardenolides have been synthesized and their biological activity has been studied. It has been established that ajmalinocardenolides possess cardiotonic and antiarrhythmic activity with a low or moderate toxicity, while cardenolides derivatives of hyoscyamine and of scopolamine have proved to be highly toxic compounds. Using fluorescent probes it has been shown that the ajmalinocardenolides are sorbed fairly strongly on liposome membranes, and they also promote the binding of calcium ions by biological membranes.

We have previously [1] reported on the production of ajmaline derivatives of strophanthidin and of hellebrigenin with cardiotonic and antiarrhythmic activity. Continuing investigations to find compounds with this type of action, we have synthesized a series of new alkaloid cardenolides (I-VII), and also an aminoantipurine derivative of strophanthidin (VIII). In planning the compounds, we started from aglycones of cardiac glycosides possessing cardiotonic activity and nitrogen compounds used in medical practice, particularly alkaloids possessing an antiarrhythmic action.

The diversity of the cardenolides used in the synthesis was due to the problem posed: to obtain valuable compounds with different ratios of the forces of cardiotonic and antiarrhythmic action. With respect to the force of their cardiotonic activity, the cardenolides taken in the synthesis form the following sequence: hellebrigenin [1] > strophanthidin > digitoxigenin > methyl strophanthidin-19-carboxylate > gitoxigenin > 17α-strophanthidin. By combining these cardenolides with the alkaloid ajmaline, compounds were obtained in which the antiarrhythmic and cardiotonic activities were correlated and also compounds in which either the cardiotonic or the antiarrhythmic activity predominated.

As before [1], the synthesis was carried out in two stages. The first consisted in obtaining intermediate halogen (haloacetyl) derivatives of cardenolides. The second was the interaction of the haloacetylcardenolides with the alkaloids ajmaline, hyoscyamine, and scopolamine with the formation of quaternary ammonium salts (I-VII); by interaction with 4-aminoantipyrine, 3β-O-(4-antipyrylaminoacetyl)strophanthidin (VIII) was obtained.

The haloacetylcardenolides were obtained by acylating the natural or transformed aglycones with bromoacetyl bromide $BrCH_2COBr$ or chloroacetyl chloride $ClCH_2COCl$.

Ajmaline reacts with haloacetylcardenolides selectively at the N(b) atom, which exhibits an increased reactivity (see [1]). From strophanthidin, 17α-strophanthidin, digitoxigenin, methyl strophanthidin-19-carboxylate and gitoxigenin were obtained, respectively: 3β-O-(ajmalin-N(b)-ioacetyl)strophanthidin chloride (I), 3β-O-(ajmalin-N(b)-ioacetyl)-17α-strophanthidin (17α-ASB) (II); 3β-O-(ajmalin-n(b)-ioacetyl)digitoxigenin) methyl 3β-O-(ajmalin-N(b)-ioacetyl)-strophanthidin-19-carboxylate bromide (IV), and 3β,16β-di-[O-ajmalin-N(b)-ioacetyl)]digitoxigenin dibromide (V).

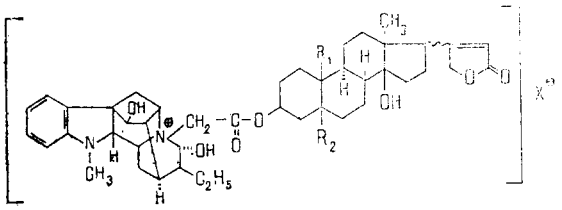
From hyoscyamine, scopolamine, on strophanthidin we also synthesized: 3β-O-(hyoscyaminoacetyl)strophanthidin (VI), 3β-O-(scopolaminoacetyl)stropanthidin (VII) and 3β-O-(4-antipyrylammonioacetyl)stropanthidin bromide (VIII). The properties of the new compounds are given on the following page:

All-Union Scientific-Research Institute of Drug Chemistry and Technology, Khar'kov.
Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 239-244, March-April, 1985. Original article submitted April 25, 1984.

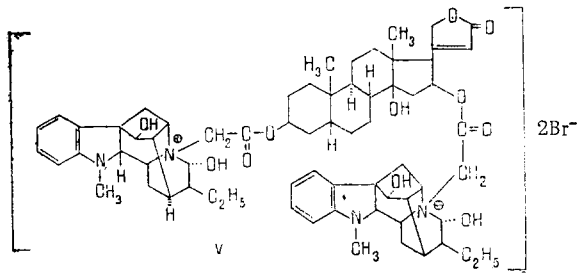
 0009-3130/85/2102-0224$09.50

Compound	Molecular formula	mp, °C	$[\alpha]_D$, deg
3β-O-(Ajmalin-N(b)-ioacetyl)stropanthidin chloride (I)	$C_{45}H_{59}O_9N_2Cl$	250-253	+43.0 ± 2 (Py)
3β-O-(Ahmalin-N(b)-ioacetyl)-17α-strophanthidin bromide (II)	$C_{45}H_{59}O_9N_2Br$	217-220	+36.1 ± 2 (Py) +71.1 ± 3 (EtOH — $CHCl_3$)
3β-O-(Ajmalin-N(b)-ioacetyl)digitoxigenin (III)	$C_{45}H_{61}O_7N_2Br$	215-218	+58.0 ± 3 (EtOH)
Methyl 3β-O-ajmalin-N(b)-ioacetylstrophanthidin-19-carboxylate bromide (IV)	$C_{46}H_{61}O_{10}N_2Br$	202-206	+81.3 ± 3 (EtOH)
3β,16β-Di-[O-(ajmalin-N(b)-ioacetyl)] - digitoxigenin dibromide (V)	$C_{67}H_{86}O_{11}N_4Br_2$	213-215	+79.0 ± 4 (EtOH)
3β-O-(Hyoscaminioacetyl)strophanthidin bromide (VI)	$C_{42}H_{56}O_{10}NBr$	168-172	−11.3 ± 2 (MeOH)
3β-O-(Scopolaminioacetyl)stropanthidin bromide (VII)	$C_{42}H_{54}O_{11}NBr$	147-149	+9.9 ± 2 (MeOH)
3β-O-(4-Antipyrylammonioacetyl)strophanthidin bromide (VIII)	$C_{36}H_{46}O_8N_3Br$	172-180	

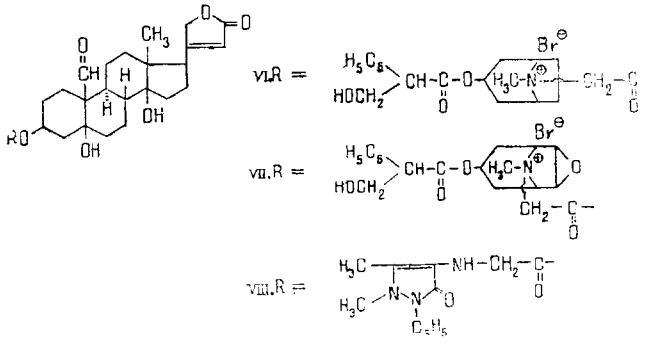
In the performance of this investigation we improved the method of obtaining 17α-cardenolides proposed previously by T. Reichstein et al. [2]. As is well known, the method consists in inverting the butenolide ring from the β to the α position by heating 17β-cardenolides in dimethylformamide in the presence of sodium tosylate and sodium acetate. According to the proposal of the original authors [2], the reaction is performed at 115°C for a day. With all the advantages of this method, there is also a substantial disadvantage consisting in the fact that cardenolides containing aldehyde groups undergo autooxidation on such lengthy



I. R_1 = CHO, R_2 = OH, связь $C_{17}-C_{20}-\beta$, X = Cl
II. R_1 = CHO, R_2 = OH, связь $C_{17}-C_{20}-\alpha$, X = Br
III. R_1 = CH_3, R_2 = H, связь $C_{17}-C_{20}-\beta$, X = Br
IV. R_1 = CH_2COOCH_3, R_2 = OH, связь $C_{17}-C_{20}-\alpha$, X = Br



V



VI. R =

VII. R =

VIII. R =

heating in the presence of the salts mentioned. This complicates the preparation of the 17α-cardenolides in the pure form and lowers their yield. In view of this fact, a somewhat modified method is proposed which consists in mixing the anhydrous cardenolide with sodium tosylate and acetate, moistening the mixture with absolute dimethylformamide, sealing it into a glass tube, and heating at 150-153°C, i.e., at a temperature close to the boiling point of the solvent, for 6-7 h. After this time, the isomerization reaction is practically complete and there are no autooxidation products.

Pharmacological investigations showed that the alkaloid cardenolides (I, II, IV, and V) exhibited pronounced cardiotonic and antiarrhythmic activity. The 17α-stropanthidin derivative (II) showed an antiarrhythmic action and an extremely weak cardiotonic action.

In the model of arrhythmia induced by calcium chloride in experiments on white rats, it was established that the use of compounds (II) and (III) in a dose of 5 mg/kg increased the survival rate of the animals by 55 and 65%, respectively, against 20% in the control. In the same arrangement of the experiment, on the administration of compound (I) in a dose of 0.1 mg/kg and of compound (IV) in a dose of 0.5 mg/kg the survival rates of the animals were 43 and 50%, respectively:

Compound (short name)	Number of animals	LD_{50} mg/kg
Ajmaline	36	140
Strophanthidin-ajmaline chloride (I)	35	130
17α-ASB (II)	30	820
Digitoxigenin-ajmaline bromide (III)	58	740
(Methyl strophanthidin-19-carboxylate)-ajmaline bromide (IV)	30	140
Strophanthidin-hyoscyamine bromide (VI)	47	27
Strophanthidin-scopolamine bromide (VII)	34	5

A study of the toxicity of the compounds obtained was performed in experiments on mice with intraperitoneal administration in comparison with ajmaline. The results showed that substances (II) and (III) have low toxicity, ajmaline and substances (I) and (IV) a moderate toxicity, and (VI) and (VII) a high toxicity in K. K. Sidorov's classification [4]. It is known that for antiarrhythmic compounds a correlation exists between their biological activity and their affinity for biomembrane lipids [5]. In view of this we made a comparative study of the affinity of 17α-ASB (II), ajmaline, stropanthidin, and the 3β-O-(ajmalin-N(b)-ioacetyl)-strophanthidin bromide (ASB) synthesized previously [1] for model phospholipid membranes (liposomes) and also of the influence of these substances on the binding of calcium ions to lipids, using the fluorescent probe method. Figure 1 shows the fluorescence spectrum of the probe 1,8-ANS (magnesium 8-anilinonaphthalene-1-sulfonate) in liposomes with and without the introduction of the compounds being studied. The intensity of the fluorescence of the probe increased substantially in the presence of ASB, and 17α-ASB, and ajmaline, which is connected with a rise in the positive charge of the membranes as a consequence of the effective sorption of these substances on them.

ASB and 17α-ASB (II) differ only by the spatial arrangement of the butenolide ring relative to the steroid skeleton. The α configuration in 17α-ASB leads to poorer binding of (II) with the membranes as compared with ASB, in which the lactone ring occupies the β position. The increase in fluorescence on the addition of ASB, as compared with ajmaline, showing the better affinity of ASB for the lipids, is obviously due to the appearance of additional points of binding in the molecule of the latter.

From the fluorescence spectra (Fig. 1) by the method of reciprocal coordinates the dissociation constant of the binding of ASB with liposomes was determined: $K_{diss} = 1.4 \cdot 10^{-4}$ M.

Since ASB possesses, in addition to an antiarrhythmic action, a moderate cardiotonic activity, we studied its influence on the capacity of phospholipid membranes for binding calcium ions. The investigation was performed in comparison with strophanthidin using a calcium-sensitive chlortetracycline probe [6]. The presence of ASB and strophanthidin led to an increase in fluorescence and, consequently, to an increase in the binding of calcium ions by phospholipids, the effects of ASB and strophanthidin being approximately the same.

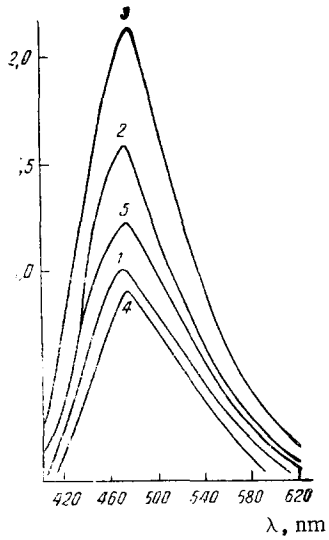


Fig. 1. Fluorescence spectra of the probe 1,8-ANS: 1) in a suspension of liposomes; 2) with 17α-ASB; 3) with ASB; 4) with strophanthidin; 5) with ajmaline.

EXPERIMENTAL

17α-Strophanthidin. A mixture of 15 g of anhydrous sodium acetate and 45 g of anhydrous sodium tosylate ground to a fine powder was placed in a thin-walled glass tube with a volume of 100 ml. A solution of 15 g of strophanthidin in 30 ml absolute dimethyl formamide was added to the tube, and then pure solvent was added until the salts has been completely wetted. The tube was sealed and was heated at 150-153°C for 7 h. After the tube had been cooled, and opened, the reaction mixture was transferred to a separatory vessel containing chloroform–ethanol (3:1) (1000 ml). The solution was washed free from salts with water and was evaporated. After three crystallizations from ethanol, 12 g of 17α-strophanthidin was obtained with mp 245-249°C; $[\alpha]_D^{20}$ +36.1 ± 2° (c 1.0; methanol).

Methyl Strophanthidin-19-carboxylate. A solution of 5 g of strophanthidin-19-carboxylic acid (obtained by the oxidation of strophanthidin with potassium permanganate as described in [3]) in 30 ml of methanol was cooled to 0-3°C and, with stirring, 50 ml of an ethereal solution of diazomethane was slowly added. The methylation process was monitored with the aid of chromatography on Silufol in the dichloromethane–methanol–water (84:15:1) system. After the end of the reaction, the solution was evaporated in vacuum. The residue, 5.2 g, consisting of methyl strophanthidin-19-carboxylate was crystallized from methanol; mp 135-140°C/177-180°C; $[\alpha]_D^{20}$ +59.1 ± 2° (c 1.0; methanol).

The procedure for obtaining the chloro- and bromoacetylcardenolides and their reaction with the alkaloids was similar to that described in [1].

Arrhythmia Model. The investigations of antiarrhythmic activity were performed on 57 white rats of both sexes weighing 120-160 g using the model of arrhythmia caused by calcium chloride. In control experiments, the administration of calcium chloride to the animals in a dose of 0.25 ml of 10% solution per 100 g body weight caused fibrillation of the ventricles in the majority of the animals, leading to their death. In the surviving animals, pronounced bradycardia and temporary cardiac arrest developed.

Determination of Acute Toxicity. The experiments were performed on 270 white mice of both sexes weighing 17-22 g. The substances was administered intraperitoneally. Observations were carried out for 7 days. The LD_{50} values were calculated by the Litchfield-Wilcox method.

Fluorescence Measurements. The fluorescent probe 1,8-ANS was excited by ultraviolet radiation at a wavelength of 365 nm, and the fluorescence maximum was measured at 475 nm. For the calcium-sensitive chlortetracycline (CTC) probe, the excitation and measurement of the fluorescence were carried out at 395 and 520 nm, respectively.

Liposomes were obtained by the ultrasonic treatment of multilayer phosphatidylcholine (egg lecithin) vesicles at a frequency of 22 kHz followed by the addition of phosphate buffer to a concentration of 0.05 M, pH 7.0.

The 1,8-ANS and CTC probes were added in the form of aqueous solutions in a concentration of $5 \cdot 10^{-6}$ M to a suspension of liposomes (0.5 mg/ml). The source of calcium ions was a $5 \cdot 10^{-3}$ M solution of calcium chloride. The final concentrations of the compounds ASB, 17α-ASB, strophanthidin, and ajmaline in the liposome suspensions studied was $2.5 \cdot 10^{-4}$ M. The fluorescence spectra were recorded on a Hitachi-4 spectrophotometer.

SUMMARY

New cardenolide derivatives of the alkaloids ajmaline, hyoscyamine, and scopolamine, and also of 4-aminoantipyrine, have been synthesized. The ajmalinocardenolides combine within themselves antiarrhythmic and cardiotonic actions with low or moderate toxicity. The hyoscyamino- and scopolaminostrophanthidins proved to be highly toxic compounds.

The ajmalinostrophanthidin bromide possessed the high affinity for phospholipid membranes that is characteristic for many antiarrhythmic agents. The presence of the cardenolide fragment in this compound caused an increase in the binding of calcium ions to liposome membranes.

LITERATURE CITED

1. I. F. Makarevich, Ya. I. Khadzhai, A. V. Nikolaeva, and V. V. Pavlova, Khim. Prir. Soedin., 537 (1979).
2. A. Kuritzkes, J. V. Euw, and T. Reichstein, Helv. Chim. Acta, 42, 1502 (1959).
3. W. A. Jacobs and M. J. Heidelberger, J. Biol. Chem., 54, 253 (1922).
4. N. F. Izmerov, I. V. Sanotskii, and K. K. Sidorov, Parameters of the Toxicometry of Industrial Poisons under the Action of a Single Dose [in Russian], Moscow (1977).
5. G. E. Dobretsov, V. V. Lyskovetsev, and N. L. Vekshin, Farmakol. Toksikol., No. 2, 136 (1979).
6. Yu. A. Vladimirov and G. E. Dobretsov, The Fluorescent Probe Method in the Investigation of Biological Membranes [in Russian], Moscow (1980).

STRUCTURE OF CUCUMARIOSIDE G_1 — A NEW TRITERPENE GLYCOSIDE FROM THE HOLOTHURIAN *Cucumaria fraudatrix*

Sh. Sh. Afiyatullov, L. Ya. Tishchenko, V. A. Stonik, A. I. Kalinovskii, and G. B. Elyakov

UDC 547.996:593.96

A new triterpene glycoside — cucamarioside G_1 — has been isolated from the Pacific Ocean holothurian *Cucumaria fraudatrix*. On the basis of physicochemical characteristics and the results of chemical transformations, its structure has been established as 16β-acetoxy-3β-[O-(3-O-methyl-β-D-xylopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-β-D-quinovopyranosyl-(1 → 2)-(4-O-sulfato-β-D-xylopyranosyloxy)]-holosta-7,24-diene.

We have previously established that the glycosidic fraction of the holothurian *Cucumaria fraudatrix* contains cucumarioside G_1 (I) and have determined the structure of the native aglycone of this glycoside as 16β-acetoxyholosta-7,24-dien-3β-ol (II) [1]. The present investigation was devoted to establishing the structure of the carbohydrate chain of cucumarioside G_1.

The acid hydrolysis of glycoside (I) gave, in addition to the aglycone (II), a mixture of D-xylose, D-quinovose, D-glucose, and 3-O-methyl-D-xylose in a ratio of 1:1:1:1, these sugars being identified by GLC in the form of acetates of the corresponding aldononitriles.

This is the first time that one of the monosaccharides, 3-O-methyl-D-xylose has been detected in hydrolysates of holothurian glycosides. We isolated this monosaccharide by prepara-

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 244-248, March-April, 1985. Original article submitted May 18, 1984.

0009-3130/85/2102-0228$09.50