GLYCOSYLATION OF CARDENOLIDES.

IV. STROPHANTHIDIN 5-α-L-RHAMNOSIDE AND 3,5-BISGLYCOSIDES

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In natural cardiac glycosides of the cardenolide series, as a rule the sugars are attached to the steroid aglycone through the C_3 hydroxy group. An exception is gomphoside [1] and glycosides similar to it in structure in which the carbohydrate components have an unusual structure and are attached by two functional groups at C_2 and C_3. In the bufadienolide series, this rule has the same force, and only recently on the basis of the NMR spectrum it was established that in scilliglaucoside and scillicyanoside — glycosides from *Scilla maritima* Backer L. — the glycoside residue is attached not to the hydroxy group at C_3, as usual, but at C_5 [2]. Partial syntheses of glycosides with the carbohydrate component at C_{12}, C_{16}, and C_{19} have been described in papers [3], [4], and [5], respectively.

Within a plan of studying the connection between structure and activity, it appeared to us to be of interest to obtain a glycoside at a tertiary hydroxy group. Although some authors have emphasized [6] that in the glycosylation of cardenolides by the Koenigs–Knorr method sugars do not add on to tertiary hydroxy groups, nevertheless, we attempted to use this method (in V. T. Chernobai's modification [7]) to obtain glycosides of strophanthidin (I) with sugar residues at C_3 and C_5.

An incidental reference to the practical possibility of such a reaction is given in a paper by Reichstein et al. where, among the usual monoglycosides of strophanthidin obtained by partial synthesis there is also mention of a compound which, according to its elementary analysis, can be assigned to diglycosides of strophanthidin. The structure of this substance was not established.

In order to establish whether the tertiary hydroxy groups of strophanthidin can be glycosylated, we first performed the condensation of strophanthidin 3-acetate (II) with acetobromorhamnose. The NMR spectrum (Fig. 1) of compound (III) isolated after the chromatographic separation of the reaction products on silica gel clearly showed in the 1.68-2.22 ppm region the signals of the protons of four acetate groups, and at 1.23 ppm the doublet (J = 6 Hz) of the methyl group of rhamnose was observed. This shows that the carbohydrate component is attached to one of the tertiary hydroxyls with the retention of the acetyl residue at C_3. Otherwise, the NMR spectrum of product (III) will have the signals only of three acetyl groups.

Under the controlled conditions with the observance of the integrity of the lactone ring, the complete saponification of the tetraacetate (III) took place with difficulty. On mild hydrolysis with potassium bicarbonate at room temperature for 13 days, compound (IV) was obtained in good yield; its NMR spectrum had a three-proton singlet of an acetyl group at 2.22 ppm, and at 5.17 ppm it was possible to see the signal of the proton geminal to this group. The IR spectrum of product (IV) showed at 1260 and 1290 cm^{-1} the split band of the asymmetric stretching vibrations of a C–O–C group, which is characteristic for a steroid acetyl group occupying an axial position [9]. Consequently, one of the acetyl groups was not saponified and, in all probability, glycoside (IV) had the structure of 5β-O-L-rhamnosylstrophanidin 3-acetate. In this case, the shift of the ester bond in the high-frequency direction (1260, 1290 cm^{-1}) is a consequence of the appearance of a hydrogen bond between the C=O of the acetyl at C_3 and one of the hydroxy groups of the rhamnose. The increased resistance to hydrolysis of the acetyl group at C_3 also finds an explanation. Here, prob-

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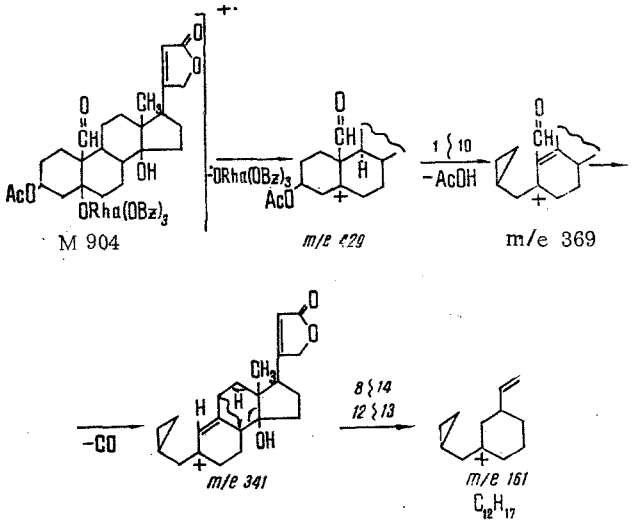
ably, there is steric hindrance arising because of the presence of the voluminous carbohydrate substitutent at C_5 [10] and the above-mentioned hydrogen bond.

The benzoate (V) that we synthesized additionally confirmed the structure proposed for compound (IV). The mass spectrum of the tribenzoate (V) had the peak of an ion with m/e 459 corresponding to the fragment $[Rha(OBz)_3 - OH]^+$. On this basis, we came to the conclusion that the acetyl group in (IV) is attached not to the sugar but directly to the aglycone. In addition, the presence in the mass spectrum of the benzoate (V) of the peak of an ion with m/e 161 [11, 12] having a carbohydrate composition unambiguously shows that the hydroxy group at C_{14} remained unsubstituted. The fragmentation of (V) can be represented in the following way:



Scheme 1

Attempts to saponify the acetyl group in (IV) under more severe conditions were unsuccessful, since under these conditions the butenolide ring opened simultaneously. Only by the prolonged (35 days at 37°C) action of an aqueous methanolic solution of potassium bicarbonate was it possible to obtain compound (VI) in relatively low yield (17.1%). There are no frequencies in the IR spectrum of the glycoside (VI) characteristic for an ester grouping, and the NMR spectrum lacks the signals of the protons of an acetyl group. When subjected to TLC [SiO_2, chloroform–methanol–water (65:35:15)], compound VI proved to be more

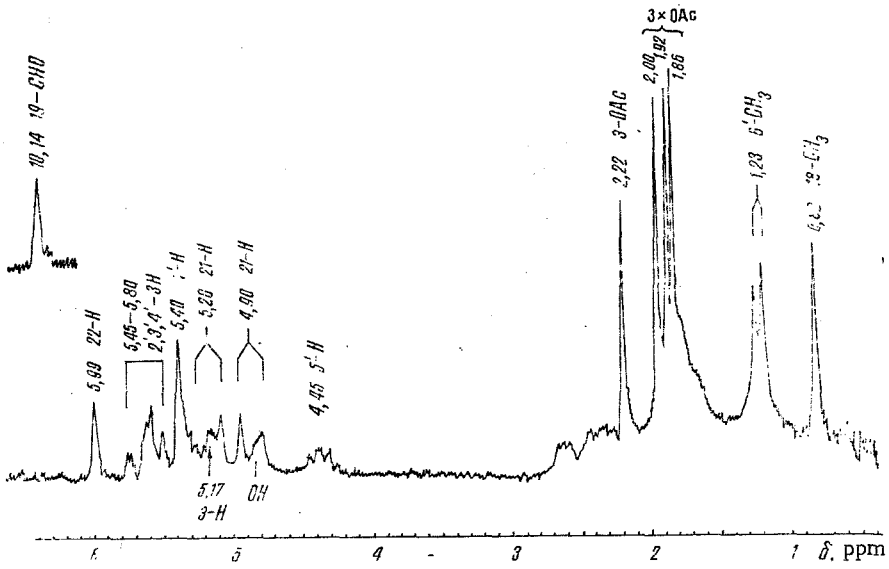
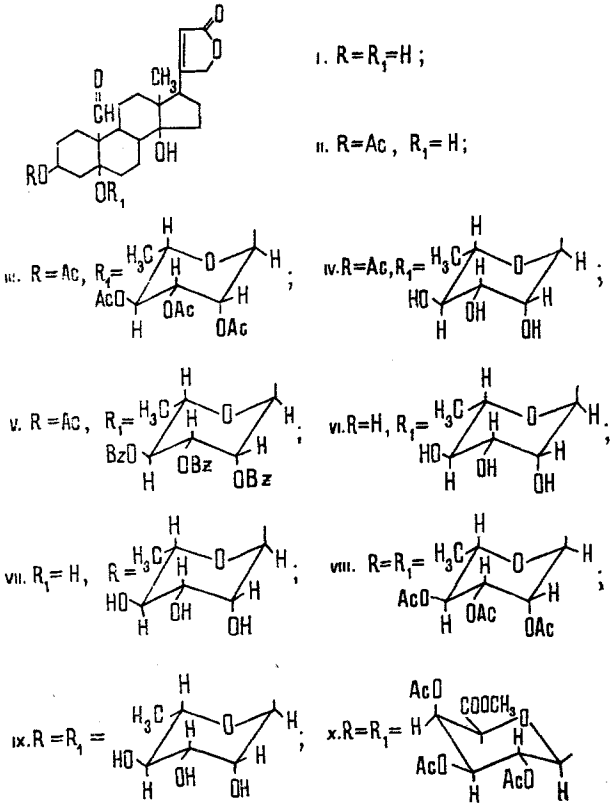


Fig. 1. NMR spectrum of 5β-O-(α-L-2',3',4'-tri-O-acetylrhamnopyranosyl)strophanthidin 3-acetate (III) (100 MHz; HMDS; δ, ppm).

polar than convallatoxin (VII) and the IR and NMR spectra of rhamnosides (VI) and (VII) could not be superimposed on one another.

The NMR spectrum of the tetraacetate (III) had the signal of a proton at $C_{5'}$, of rhamnose in the 4.45-ppm region with $W_{1/2}$ = 17 Hz, which shows the 1C conformation. Calculation by the method of molecular rotation differences [13] ($[M]_D$ (VI) −198°; $[M]_D$ (I) +170°; $\Delta[M]_D$ −368°) shows that the sugar residue is attached to the aglycone by an α bond [14]. Thus, (VI) is 5β-O-(α-L-rhamnopyranosyl)-3β,14β-card-20(22)-enolide. For brevity, it may be called 5-isoconvallatoxin.



Scheme 2

The condensation of strophanthidin (I) with acetobromorhamnose followed by chromatography of the reaction products gave the hexaacetate (VIII), the saponification of which with ammonia in methanol yielded strophanthidin 3,5-bisrhamnoside (IX). The NMR spectrum of compound (IX) confirmed the presence of two rhamnose residues. The broadened singlets of the equatorial anomeric protons did not overlap one another and appeared clearly at 5.28 ppm (for the rhamnose at C_3) and at 5.42 ppm (for the rhamnose at C_5).

The bifunctional glycoside (X) was obtained similarly. We made the assignment of the second sugar residue in compound (VIII) and (X) to C_5 on the basis of experimental facts obtained in the determination of the structure of 5-isoconvallatoxin (VI). In our view, the extrapolation is justified.

The biological activity of the compounds obtained will be considered separately.

EXPERIMENTAL

For thin-layer chromatography (TLC) we used type KSK silica gel with 5% of gypsum. Crystalline 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide and crystalline methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-galacturonate were obtained under the conditions described in the literature [15, 16], and silver carbonate was obtained directly before use by Becker's method [17]. Before use, the dichloroethane was distilled twice over $CaCO_3$ and twice over $CaCl_2$, and toluene was distilled twice over sodium. The UV spectra were taken on a SF-4 spectrophotometer, the IR spectra on a UR-20 instrument and the NMR spectra on a JNM-4H-100 instrument (100 MHz, HMDS, δ, ppm). Arbitrary symbols: br. s) broadened singlet; d) doublet; q) quartet; m) multiplet.

General Method of Performing Condensations. A solution of the aglycone (1:30) in a mixture of dichloroethane and toluene (5:1 by volume) was heated with stirring at the boiling point of the mixture. After the first 10 ml of solvents had been distilled off, calcium oxide and one fifth of the required amount of silver carbonate were added to the reaction mixture. Then a solution of the acetobromo sugar (1:10) in a mixture of dichloroethane and toluene was added dropwise at such a rate that the volume of the reaction mixture remained constant. The remainder of the silver carbonate was added periodically. The reaction mixture was boiled with the distillation of the solvent used, and new portions of dichloroethane and toluene in a ratio of 1:1 were added. The course of the reaction was monitored by TLC in the ethyl acetate–benzene (9:1) system. After the amount of reaction product had ceased to increase, judging by TLC, the reaction mixture was filtered, the precipitate on the filter was washed several times with chloroform, and the solution was evaporated in vacuum at 45°C to dryness. The residue was chromatographed on silica gel, the column being eluted with benzene containing gradientwise increasing concentrations of ethyl acetate. Since the ratio of the substances taken in the reaction, the time required for the addition of the acetobromosugar, and the total time of the reaction were different in all the experiments, this information will be given separately.

5β-O-(α-L-2',3',4'-Tri-O-acetylrhamnopyranosyl)strophanthidin 3-Acetate (III) from (II). The reaction was performed with 10.5 g (23.5 mmole) of strophanthidin acetate (II), 10 g of CaO, and 44 g of Ag_2CO_3. A solution of 50 g (141.6 mmole) of acetobromorhamnose was added in the course of one hour. The total reaction time was 7 h. The product obtained was crystallized from ethanol. The yield of (III) ($C_{37}H_{50}O_{14}$) was 1.69 (10%). The (III) synthesized had mp 243°C, $[\alpha]_D^{22}$ +10.5 ± 3° (c 1.10; chloroform), $\lambda_{max}^{C_2H_5OH}$, nm: 217 nm (log ε 4.30); ν_{max}^{KBr}, cm^{-1}; 3520 (OH), 2740 (CHO), 1760, 1632 (butenolide ring), 1730, 1260, 1240 (acetyl). NMR spectrum (C_5D_5N), ppm: 0.82 (3H at C_{18}, s), 1.23 (3H at C_6', d, J = 6 Hz), 1.86, 1.92, 2.00 (9H of 3 Ac at C_2', C_3', C_4', s) 2.22 (3H of Ac at C_3, s), 4.45 (H at C_5', m), 4.90 and 5.20 (2H at C_{21}, q, centers of doublets, J = 18 Hz), 5.17 (H at C_3, m), 5.40 (H at C_1', br. s), 5.45-5.80 (3H at C_2', C_3', and C_4', m), 5.99 (H at C_{22}, s), and 10.14 (H at C_{19}, s).

5β-O-(α-L-Rhamnopyranosyl)strophanthidin 3-Acetate (IV) from (III). A solution of 10.8 g of $KHCO_3$ in 450 ml of water was added to a solution of 1.08 g of the acetate (III) in 1 liter of methanol, and the mixture was left at room temperature in an atmosphere of nitrogen for 13 days. Then the methanol was distilled off and the residual aqueous solution was extracted successively with ether (5 × 100 ml) and chloroform–ethanol (9:1) (10 × 200 ml). The chloroform–ethanol extract was evaporated to dryness and the residue was crystallized from methanol. Yield 0.73 g (82%). After recrystallization from methanol, compound (IV), $C_{31}H_{44}O_{11}$, had mp 235-238°C, $[\alpha]_D^{29}$ +6.3 ± 3° (c 0.86; chloroform; $\lambda_{max}^{C_2H_5OH}$, nm: 216 (log ε 4.19); ν_{max}^{KBr}, cm^{-1}: 3410-3520 (OH), 2740, 1710 (CHO), 1780, 1750, 1630 (butenolide ring), 1290, 1260 (C–O–C). NMR spectrum (C_5D_5N), ppm: 0.81 (3H at C_{18}, s), 1.44 (3H at C_6', d, J = 7 Hz), 2.23 (3H of Ac at C_3, s), 4.85, 5.15 (2H at C_{21}, q, centers of doublets, J = 18 Hz), 5.18 (H at C_3, m), 5.40 (H at C_1', br. s), 6.00 (H at C_{22}, s), 9.98 (H at C_{19}, s).

5β-O-(α-L-2',3',4'-Tri-O-benzoylrhamnopyranosyl)strophanthidin 3-Acetate (V) from (IV). A solution of 25 mg of the glycoside (IV) in 0.5 ml of absolute pyridine was treated with 0.065 ml of benzoyl chloride and the mixture was left at 0°C for 2 h. Then it was left at room temperature for another 24 h, 0.1 ml of methanol was added, and after another two hours the mixture was evaporated to dryness, the residue was dissolved in chloroform, the solution was washed successively with 2 N HCl, 2 N Na_2CO_3, and water, and the chloroform was evaporated off. The residue was chromatographed on silica gel. Methyl benzoate was eluted with a mixture of benzene and chloroform, and the reaction product (V) with chloroform. The dry residue from the chloroform eluate was crystallized from acetone–ether, which gave 23 mg of substance (V) yield 60.1%. Compound (V), $C_{52}H_{56}O_{14}$, had mp 218-226°C, $[\alpha]_D^{29}$ +81.9 ± 2° (c 0.83; chloroform); ν_{max}^{KBr}, cm^{-1}: 3580 (OH), 2750, 1715 (CHO), and 1780, 1750, 1630 (butenolide ring), 1730, 1285, 1280, 1255 (ester grouping), 1610, 1590, 720 (aromatic ring). Mass spectrum, m/e (%): 459 (0.1, 369 (0.1) 351 (0.1), 341 (0.9), 322 (0.2), 308 (0.1), 215 [0.3; Rha(OBz)$_3$–2 BzOH], 161 (0.4), fragments of benzoic acid 122 (16.2) and 105 (100).

5β-O-(α-L-Rhamnopyranosyl)strophanthidin (VI) from (IV). A solution of 1.23 g of glycoside (IV) in 900 ml of methanol was added to a solution of 9 g of $KHCO_3$ in 400 ml of water. The reaction was performed in an atmosphere of nitrogen at 37°C for 35 days. The methanol was distilled off and the residue was extracted with ether (6 × 100 ml), with chloroform

(6 × 200 ml), and with mixtures of chloroform and ethanol in ratios of 9:1 (6 × 200 ml) and 4:1 (10 × 200 ml). The chloroform and chloroform–ethanol (9:1) extracts were evaporated, and the initial (IV) was crystallized from methanol. The total recovery of (IV) that had not reacted (see below) was 520 mg (42.3%). The chloroform–ethanol (4:1) extract was evaporated to dryness and the small amount of contaminating (IV) was eliminated by crystallization from methanol. The mother solution was concentrated, and 190 mg (17.1%) of substance was obtained. Compound (VI), $C_{29}H_{42}O_{10}$, after recrystallization from ethanol–benzene, had mp 210-215°C, $[\alpha]_D^{22}$ −3.6 ± 3° [c 0.54; chloroform–methanol (1:1)]; $\lambda_{max}^{C_2H_5OH}$, nm: 217 (log ε 4.25); ν_{max}^{KBr}, cm^{-1}: 3400-3500 (OH), 2780, 1712 (CHO), 1815, 1757, 1622 (butenolide ring). NMR spectrum (C_5D_5N), ppm: 0.86 (3H at C_{18}, s), 1.45 (3H at C_6', d, J = 7 Hz), 4.87, 5.17, (2H at C_{21}, q, centers of doublets, J = 18 Hz), 5.48 (H at C_1', br. s), 6.00 (H at C_{22}, s), 9.98 (H at C_{19}, s).

3β,5β-O-Bis(α-L-2',3',4'-tri-O-acetylrhamnopyranosyl)strophanthidin (VIII) from (I). A solution of 42 g (118.9 mmole) of acetobromorhamnose was added over 2 h to a mixture of 5 g (12.3 mmole) of strophanthidin (I), 8 g of CaO, and 40 g of Ag_2CO_3. The total reaction time was 4.5 h. This yielded 1.59 g of the acetate (VIII) and 159 mg of convallatoxin acetate. The yield of compound (VIII) was 13.6%. After recrystallization from methanol, compound (VIII) $C_{47}H_{64}O_{20}$, had mp 163-170°C (decomp.), $[\alpha]_D^{25}$ −1.9 ± 3° (c 0.83; chloroform); $\lambda_{max}^{C_2H_5OH}$, nm: 217 (log ε 4.28); ν_{max}^{KBr}, cm^{-1}: 3480-3570 (OH), 2760 (CHO), 1785, 1760, 1630 (butenolide ring), 1745, 1250-1220 (acetyl).

Convallatoxin (VII). By treatment with ammonia in methanol, 159 mg of convallatoxin acetate yielded 92 mg (1.4%) of (VII). After recrystallization from aqueous methanol, compound (VII), $C_{29}H_{42}O_{10}$, had mp 222-224°C, $[\alpha]_D^{22}$ 0 ± 3° (c 1.0; ethanol [18]. NMR spectrum (C_5D_5N), ppm: 0.8 (3H at C_{18}, s), 1.45 (3H at C_6', d, J = 6 Hz), 4.85, 5.15 (2H at C_{21}, q, centers of doublets, J = 18 Hz), 5.31 (H at C_1', br. s), 5.98 (H at C_{22}, s), 10.09 (H at C_{19}, s).

3β,5β-O-Di(α-L-rhamnopyranosyl)strophanthidin (IX) from (VIII). A solution of the hexaacetate of (VIII) (0.43 g) in 18 ml of absolute methanol was treated with 5.5 ml of methanol saturated with ammonia. After 15 h, the methanol was distilled off in vacuum. The residue was recrystallized from ethanol, giving 0.29 g (96.8%) of compound (IX). The bisglycoside (IX), $C_{35}H_{52}O_{14}$, had mp 228-231°C (decomp.), $[\alpha]_D^{25}$ −7.1 ± 3° (c 1.68; methanol–chloroform (1:1)); $\lambda_{max}^{C_2H_5OH}$, nm: 217 (log ε 4.22); ν_{max}^{KBr}, cm^{-1}: 3300-3500 (OH), 2740, 1710 (CHO), 1815, 1780, 1755, 1740, 1625 (butenolide ring). NMR spectrum (C_5D_5N), ppm: 0.83 (3H at C_{18}, s), 1.37 (3H at C_6'', d, J = 6 Hz),* 1.50 (3H at C_6', d, J = 6 Hz), 4.80, 5.20 (2H at C_{21}, q, centers of doublets, J = 18 Hz), 5.28 (H at C_1', br. s), 5.42 (H at C_1'', br. s), 5.99 (H at C_{22}, s), 9.97 (H at C_{19}, s).

Strophanthidin 3β,5β-Bis-O-(methyl 2',3',4'-tri-O-acetyl-β-D-galactopyranosiduronate) (X) from (I). The reaction mixture consisted of 1 g (2.47 mmole) of strophanthidin (I), 2.5 g of Ag_2CO_3, 0.5 g of $CaSO_4$, 0.5 g of CaO, 0.1 g of activated carbon, and solvent. Over 2 h, 1.8 g (4.78 mmole) of the acetobromo derivative of methyl galacturonate was added. The total reaction time was 5 h. The product was crystallized from ethanol to give 250 mg (10%) of the hexaacetate (X), $C_{49}H_{64}O_{24}$ with mp 223-226°C, $[\alpha]_D^{22}$ +8.0 ± 3° (c 1.90; chloroform); $\lambda_{max}^{C_2H_5OH}$, nm: 217 (log ε 4.22); λ_{max}^{KBr}, cm^{-1}: 3500-3550 (OH), 1760 (C=O), 1635 (double bond of a butenolide ring), 1245 (C–O–C). NMR spectrum ($CDCl_3$), ppm: 0.76 (3H at C_{18}, s), 2.0-2.35 (18H of 6Ac), 3.75 (3H in OCH_3, s), 3.80 (3H in OCH_3, s), 4.45 (H at C_3 and H at C_5'), 5.75 (2H at C_4' and C_4'', m), 5.84 (H at C_{22}, s), 9.57 (H at C_{19}, s). The subsequent fractions yielded 1.19 g (66.6%) of methyl (strophanthidin 3β-O-2',3',4'-tri-O-acetylgalactopyranosid)uronate [19].

SUMMARY

1. The Koenigs–Knorr condensation of acetobromorhamnose with strophanthidin acetate has given strophanthidin L-rhamnoside substituted at the C_5 hydroxyl, and its condensation with free strophanthidin has given strophanthidin di-α-O-rhamnoside substituted at the C_3 and C_5 hydroxy groups.

2. Under similar conditions, methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-galacturonate and strophanthidin have given the corresponding strophanthidin diglycoside substituted at the C_3 and C_5 hydroxyls.

*The double primes denote the signals of the protons of the sugar residue at C_5.

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QUANTITATIVE DETERMINATION OF THE ALKALOIDS OF *Anabasis aphylla* BY THIN-LAYER CHROMATOGRAPHY

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In connection with the proposal of a new method for the separate isolation of the alkaloids of *Anabasis aphylla* [1] with the subsequent production of pachycarpine from the high-boiling fraction of the alkaloids [2], the necessity has arisen for the development of a method for the quantitative determination of the main alkaloids in the raw material, the intermediates, and the final products of this process.

Several methods exist for the quantitative determination of anabasine in the raw material and in the products of anabasine production [3]. According to the well-known standard method (factory method) [4], the anabasine is precipitated in the form of a complex mercury salt and, after decomposition with hydrochloric acid, the alkaloid is titrated with tungstosilicic acid. When the anabasine is precipitated in the form of the mercury complex, some other accompanying alkaloids (aphylline, aphyllidine, anabasamine) may also be precipitated, which is responsible for the high results of the standard method.

The present paper gives a method for the quantitative determination of the main alkaloids of Anabasis by means of thin-layer chromatography. The chromatography of the alkaloids has been performed in the acetone–water (100:8) (1) and the ether–chloroform (100:70) (2) systems in a non-fixed layer of alumina (activity grade II according to Brockmann). Af-

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