Strophanthidin Cardenolides Containing Hexoses of the Mannose Series[1a]

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Employing a modified Koenigs-Knorr type synthesis, strophanthidin (VI) was coupled with tetra-O-acetyl-α-D-mannosyl bromide (I) and with tri-O-benzoyl-α-D-rhamnosyl bromide (II) to give, respectively, after saponification of the reaction products, strophanthidin α-D-mannopyranoside (VII) and strophanthidin α-D-rhamnopyranoside (VIII), the latter of which was converted to a crystalline tri-O-acetyl derivative VIIIa. Both cardenolides VII and VIII have an "unnatural" glycosidic linkage, when compared with natural D-hexosides of strophanthidin having the β-anomeric configuration, and show a low order of cardiotonic activity. The non-naturally occurring β-L-mannose (III) was converted to penta-O-acetyl-β-L-mannose (IV) which reacted with hydrogen bromide–acetic acid to give tetra-O-acetyl-α-L-mannosyl bromide. The latter coupled with strophanthidin (VI) to give, after removal of the protecting groups, strophanthidin α-L-mannopyranoside (6′-hydroxyconvallatoxin) (IX) which was converted into an amorphous tetra-O-acetyl derivative IXa. The L-mannoside IX has a potency (MLD 0.069 mg. kg.$^{-1}$) superseding that of convallatoxin (6-deoxy-α-L-mannopyranoside of strophanthidin) and is, therefore, the most potent of all known cardenolides. These results support our postulate that deoxygenation in the carbohydrate component of a cardenolide leads to a loss in potency.

According to Klyne,[2] cardenolides of natural origin containing sugars of the D-series are invariably β-anomers while those which contain L-sugars have the α-anomeric configuration. Previously we reported[3] the synthesis of the α-digitoxoside (2,6-dideoxy-α-D-*ribo*-hexoside)[3a] and the α-D-rhamnoside (6-deoxy-α-D-mannoside)[3b] of digitoxigenin (3β,14β-dihydroxy-5β-card-20(22)-enolide); both possess an "unnatural" glycosidic linkage and show surprisingly low potencies when compared with assay values obtained for seven hexosides of digitoxigenin of natural origin.[4] This observation suggested to us that, in general, the presence of an α-glycosidic linkage in cardenolides containing D-sugars provided for a molecular conformation unsatisfactory for optimum cardiotonic activity. In order to lend support to this contention, we undertook the preparation of two additional cardenolides, each to contain a D-hexose and to possess an α-glycosidic linkage. Prior to the work described in this paper the naturally occurring convallatoxin [6-deoxy-α-L-mannoside of strophanthidin (VI)] had been considered to be the most potent of all cardenolides and, because of this, we chose to prepare glycosides of strophanthidin (3β,5β,14β-trihydroxy-19-oxocard-20(22)-enolid) (VI) containing D-mannose on the one hand and 6-deoxy-D-mannose (D-rhamnose[5]) on the other.

Recent work[6] in this Laboratory has yielded information which now leaves little doubt that deoxygenation in the pyranose is, in general, unfavorable for cardiotonic activity. According to this, the reverse should be true and by "oxygenating" the sugar component of a naturally occurring cardenolide, one should effect an increase in potency. With this in mind, we undertook to increase the oxygen function of the carbohydrate component of the potent convallatoxin by converting the C-5 methyl group to a hydroxymethyl group; accordingly such a change should result in a substantial increase in potency.

Stable O-acylglycosyl halides prepared from hexoses of the mannose series (to include D-rhamnose) are invariably α-anomers and, owing to the fact that the C-2 acyloxy group is an axial substituent, such derivatives are at the same time 1,2-*trans* halides. Under specified conditions such halides may undergo displacement at C-1 with net *retention* of configuration[7]; in fact with each of all previously reported syntheses of cardenolides involving 1,2-*trans* halides in the presence of silver carbonate, an α-glycoside was formed to exclude both the alternate β anomeric form and an orthoester. It should follow therefore that mannosyl halides (as well as rhamnosyl halides) should give rise to α-cardenolides, irrespective of which enantiomeric form of the sugar is involved. In the present study, three new cardenolides were prepared and the anomeric configuration of each was shown to be α, as determined by application of Klyne's rule of molecular rotational additivities.[2]

The known 2,3,4,6-tetra-O-acetyl-α-D-mannosyl bromide (I)[8] was coupled with strophanthidin (VI) in the presence of silver carbonate, using an azeotropic distillation procedure essentially the same as that described by Meystre and Miescher.[9] The O-acylated cardenolide was not isolated; instead the reaction mixture was saponified *in toto*, thus rendering all extraneous carbohydrate material water soluble. Extraction with organic solvents gave the desired 3β-(α-D-mannopyranosyl)-5β,14β-dihydroxy-19-oxocard-20(22)enolide (VII) in 20% yield.

By a similar procedure, strophanthidin (VI) was treated with 2,3,4-tri-O-benzoyl-α-D-rhamnosyl bromide (II)[5b] and, after saponification of the reaction mixture, there was obtained in 56% yield, 3β-(6-deoxy-α-D-mannopyranosyl)-5β,14β-dihydroxy-19-oxocard-20-(22)-enolide (VIII), which afforded a crystalline tri-O-acetylated derivative (VIIIa).

(1) (a) This work was supported largely by U. S. Public Health Service Grant H-5839; (b) to whom all enquiries regarding this paper should be addressed; (c) post-doctoral Research Associate, Georgetown University.

(2) W. Klyne, Proc. Biochem. Soc., 288th Meeting, *Biochem. J.*, **47**, xli (1950).

(3) (a) W. W. Zorbach and T. A. Payne, *J. Am. Chem. Soc.*, **82**, 4979 (1960); (b) W. W. Zorbach, G. D. Valiaveedan, and D. V. Kashelikar, *J. Org. Chem.*, **27**, 1766 (1962).

(4) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 801.

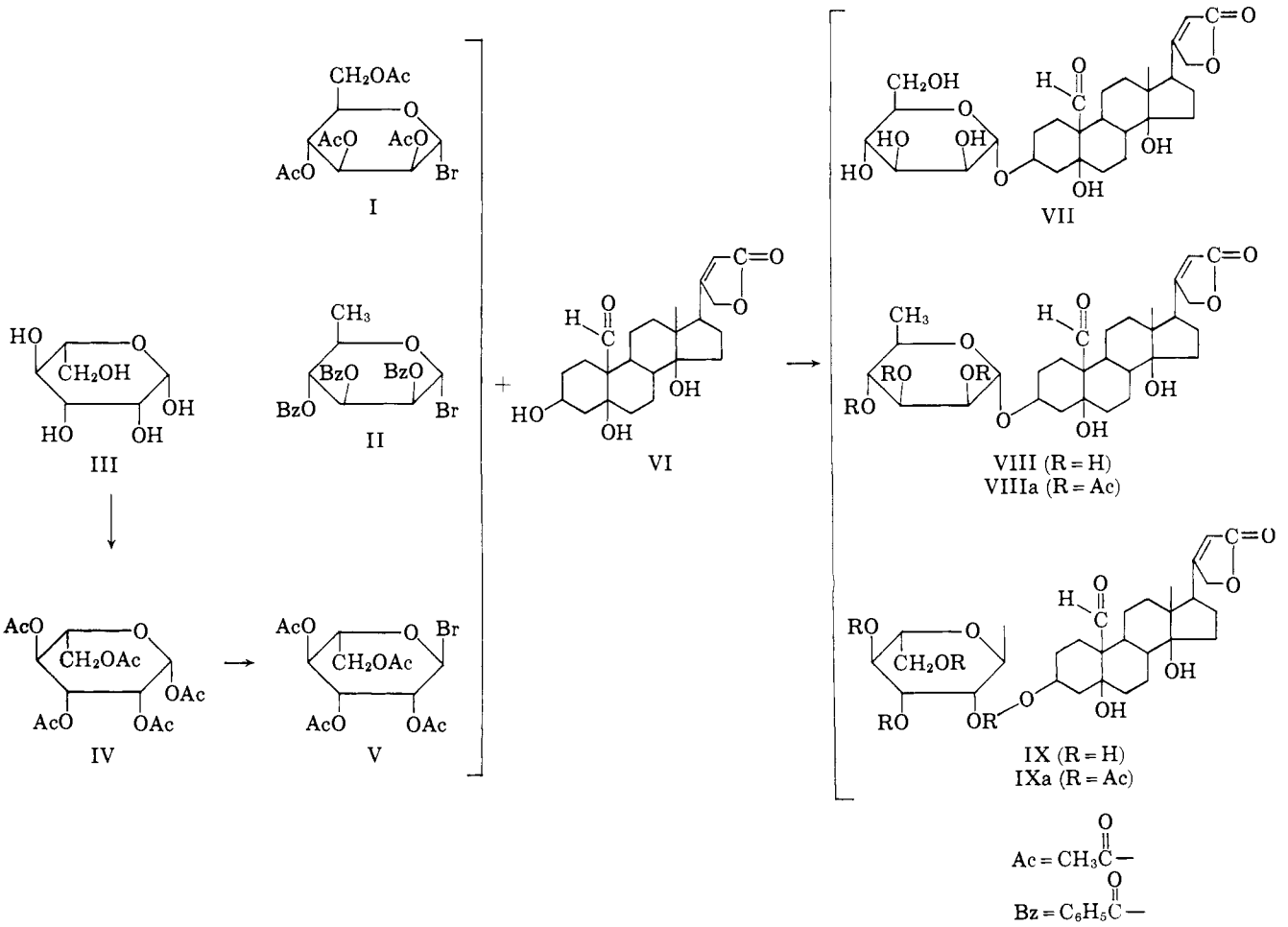
(5) (a) W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **68**, 628 (1946); (b) W. W. Zorbach and C. O. Tio, *J. Org. Chem.*, **26**, 3543 (1961).

(6) (a) W. W. Zorbach and G. Pietsch, *Ann. Chem.*, **655**, 26 (1962); (b) W. W. Zorbach and W. Buhler, *ibid.*, in press.

(7) A. Thompson and M. L. Wolfrom in "The Carbohydrates," W. Pigman, ed., Academic Press, Inc., New York, N. Y., 1957, p. 156.

(8) E. A. Talley, D. H. Reynolds, and W. L. Evans, *J. Am. Chem. Soc.*, **65**, 577 (1943).

(9) C. H. Meystre and K. Miescher, *Helv. Chim. Acta*, **27**, 231 (1944).



In order to increase the oxygen function of the carbohydrate component of the potent convallatoxin it was necessary to work with the non-naturally occurring L-mannose, for which an excellent synthesis is provided by Sowden and Fischer.[10] In contrast to the experience of these authors, however, we found that the β-L-mannose (III) thus prepared crystallized with considerable difficulty. When III was acetylated under the usual conditions at 0°, 1,2,3,4,6-penta-O-acetyl-β-L-mannose (IV) was obtained in 51% yield. Treatment of IV with hydrogen bromide–acetic acid gave 91% of 2,3,4,6-tetra-O-acetyl-α-L-mannosyl bromide (V) which coupled with strophanthidin (VI) to give, after saponification of the reaction products, 24% of 3β-(α-L-mannopyranosyl)-5β,14β-19-oxocard-20(22)-enolid (6′-hydroxyconvallatoxin) (IX).[11] The structure of the new cardenolide IX was given support by conversion to the tetra-O-acetylated derivative IXa. Although IXa could be obtained only as an amorphous powder a satisfactory elemental analysis was obtained.

Assay results given in Table I for the two "unnatural" α-D cardenolides VII and VIII show a low order of potency when compared with the naturally occurring convallatoxin as well as with other natural monosides of strophanthidin,[4] and are qualitatively in agreement with our findings with the α-digitoxoside[3a] and the α-D-rhamnoside[3b] of digitoxigenin. On the other hand 6′-hydroxyconvallatoxin (IX), although an α-glycoside, possesses a "natural" glycosidic linkage owing to the fact that it contains an L-hexose. As anticipated, the additional oxygen function at C-6 of the carbohydrate component has increased significantly the potency (especially the molar potency) of IX beyond that of convallatoxin, and it follows therefore that 6′-hydroxyconvallatoxin (IX) displaces convallatoxin from its long held position as the most potent of all cardenolides.

TABLE I

Hexosides of strophanthidin	LD/mg.[a]	LD/μmole
α-L-Rhamnopyranoside (convallatoxin)	12.6	7.0
α-D-Rhamnopyranoside[b] (VIII)	7.2	4.0
α-L-Mannopyranoside (IX)[b] (6′-hydroxyconvallatoxin)	14.5	8.2
α-D-Mannopyranoside (VII)[b]	3.9	2.2

[a] LD/mg. refer to the number of lethal doses per mg. and may be obtained simply by taking the reciprocal of the MLD [mean (geometric) lethal dose in mg./kg., as measured in 10 cats] for a given cardenolide. This method of expression, recently inaugurated by Dr. K. K. Chen, Eli Lilly and Co., Indianapolis, Indiana, and privately communicated to the authors, is to be commended in that larger values bespeak of higher potencies, and *vice versa*. [b] The authors are much indebted to Drs. K. K. Chen and Francis G. Henderson, Eli Lilly and Company, Indianapolis, Indiana, for carrying out the assays of the three new cardenolides.

Experimental

All melting points were determined using a Kofler hot stage.

General Procedure for Coupling Reactions.—To a 100-ml. two-neck flask fitted with a dropping funnel and condenser is delivered a solution of 500 mg. (1.23 mmoles) of strophanthidin (VI) in 20 ml. of anhydrous 1,2-dichloroethane. To this solution is added 700 mg. (2.53 mmoles) of dry, freshly prepared silver

(10) J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **69**, 1963 (1947).

(11) The preparation of 6-hydroxyconvallatoxin (IX) has been reported summarily as a "KOM" in *Naturwiss.*, 50/3, 93 (1963).

carbonate. With efficient magnetic stirring, the flask and contents are heated by means of an oil bath and approximately one-half of the solvent is allowed to distil over at a moderate rate. Next, a solution of 2.5 mmoles of the sugar halide (I, II, or V) in 100 ml. of anhydrous 1,2-dichloroethane is delivered to the dropping funnel and this solution is added dropwise to the stirring mixture over a period of 3.5–4.5 hr., during which time the solvent from the reaction flask is distilled over at an equal rate. In this manner, the volume of the solvent in the reaction flask is kept constant.

After completion of the addition of the bromide, an additional 50 ml. of anhydrous 1,2-dichloroethane is added dropwise, with distillation maintained as in the foregoing. The insoluble silver salts are filtered and the filtrate is evaporated *in vacuo* at 30°. The sirupy residue is dissolved in 200 ml. of methanol and to this solution is added 65 ml. of water containing 1.4 g. of potassium bicarbonate. After stirring magnetically at room temperature for 10 days, the solution is concentrated *in vacuo* at 30° to a volume of *ca.* 60 ml. Details for the isolation of the cardenolides VII, VIII and IX are to be found in the subsequently described individual experiments.

3β-(α-D-Mannopyranosyl)-5β,14β-dihydroxy-19-oxo-card-20(22)-enolide (VII).—The coupling of strophanthidin (VI) with 2,3,4,6-tetra-O-acetyl-α-D-mannosyl bromide (I) and saponification of the reaction products according to the general procedure given resulted in 60 ml. of aqueous solution which was extracted successively with ether, chloroform, chloroform–ethanol (9:1, 9:2, and 9:3), employing three-300 ml. portions of each solvent. From the chloroform–ethanol (9:1 and 9:2) extracts, there was obtained impure material which, when recrystallized 4 times from ether–methanol, gave pure cardenolide VII, m.p. 259–263°, $[\alpha]^{18}D$ +78.7° (*c*, 0.419, 90% methanol), λ_{max}^{EtOH} 217 mμ (4.29). Compound VII gave a positive Kedde test and when chromatographed on paper by an ascending technique, employing 1-butanol saturated with water, had R_f 0.31.

Calcd. for [M][strophanthidin (VI)] + [M][methyl α-D-mannopyranoside][12]: +17400° + 17850° = +35250°. Calcd. for [M][strophanthidin (VI)] + [M][diethyl β-D-mannopyranoside][12]: +17400° − 16490° = +910°. Found for [M] [strophanthidin-α-D-mannopyranoside (VII)]: +44100°. The glycosidic linkage in VII has therefore the α-configuration.

Anal. Calcd. for $C_{29}H_{42}O_{11}$: C, 61.46; H, 7.47. Found: C, 61.30; H, 7.59.

3β-(6-Deoxy-α-L-mannopyranosyl)-5β,14β-dihydroxy-19-oxo-5β-card-20(22)-enolide (VIII).—The coupling of 405 mg. (1 mmole) of strophanthidin (VI) with 1080 mg. (2 mmoles) of 2,3,4-tri-O-benzoyl-α-D-rhamnosyl bromide (II) and saponification of the reaction mixture as described (*vide supra*) resulted in 80 ml. of aqueous solution which was extracted successively with three-200 ml. portions each of ether, chloroform, and chloroform–ethanol (9:1). From the chloroform–ethanol (9:1) extracts there was obtained 410 mg. of crude material which, when recrystallized from methanol, gave 310 mg. (53%) of the D-rhamnoside (VIII) as a dihydrate, m.p. 249–254°. By drying overnight at 110° (0.1 mm.) VIII was obtained as anhydrous material, and when placed on a hot stage preheated to 250°, melted at 261–263°. $[\alpha]^{15}D$ +95.8° (*c* 0.855, methanol), λ_{max}^{MeOH} 217 mμ (4.27). Compound VIII gave a positive Kedde test and when chromatographed on paper treated with the reverse phase by a descending technique, employing 1-butanol saturated with water, had R_f 0.76.

Calcd. for [M][strophanthidin (VI) + methyl α-D-rhamnopyranoside[13]]: +17,400° + 10,900° = +28,300°. Calcd. for [M][strophanthidin (VI) + methyl β-D-rhamnopyranoside[13]]: +17,400° − 17,240° = +160°. Found for [M] [strophanthidin-α-D-rhamnopyranoside (VIII)]: +52,700°. The glycosidic linkage in VIII has there the α-configuration.

Anal. Calcd. for $C_{29}H_{42}O_{10}$: C, 63.25; H, 7.69. Found: C, 63.49; H, 7.88.

Tri-O-acetyl Derivative VIIIa.—To a solution of 1 ml. of acetic anhydride in 1.5 ml. of pyridine was added 100 mg. (0.17 mmole) of the cardenolide dihydrate VIII. After standing at room temperature for 24 hr., the solution was dissolved in chloroform–ether (1:3) and was extracted in turn with *N* sulfuric acid and water. After drying over magnesium sulfate the extract was evaporated *in vacuo* and the oily residue was dissolved in acetone, decolorized with Darco G-60, and was concentrated to *ca.* 5 ml. Crystalline material separated on the addition of ether; this was recrystallized once from acetone–ether, giving pure VIIIa, m.p. 162–164° (dec.). $[\alpha]^{23}D$ +73.9° (*c* 0.886, CH_2Cl_2). For analytical purposes the material was dried overnight at 80° (0.1 mm.).

Anal. Calcd. for $C_{35}H_{48}O_{13}$: C, 62.12; H, 7.15. Found: C, 61.97; H, 7.37.

β-L-Mannose (III).—The sugar III was synthesized following closely the directions of Sowden and Fischer.[10] Higher yields from its phenylhydrazone were obtained when the latter was brought to a high state of purity; this was accomplished by decolorizing the hydrazone with Darco G-60 followed by recrystallization. After treatment of the phenylhydrazone with benzaldehyde and and extraction with chloroform to remove the benzaldehyde phenylhydrazone and unreacted benzaldehyde, the aqueous solution was immediately deionized with Amberlite MB-1 ion exchange resin. Even this treatment did not allow for ready crystallization and only by adding water in increments of 0.5% to absolute ethanol did we arrive at a solvent which yielded crystalline material. When water–ethanol (2.5–97.5) was employed, the β-L-mannose (III) crystallized, but required 5–7 days to do so; even then it amounted to only 50–60% of the sirupy material obtained each time. M.p. 130–132°, $[\alpha]^{25}D$ +11.9° (7 min.) → −11.8° (equil.) (*c* 2.37, water).

1,2,3,4,6-Penta-O-acetyl-β-L-mannose (IV).—To a stirring solution of 2.5 ml. of acetic anhydride in 3.2 ml. of pyridine at 0° was added, in small portions, 500 mg. (2.8 mmoles) of finely powdered β-L-mannose. After standing for 24 hr. at 5°, the mixture was poured into ice water and, after standing for several hr., the sirupy material which separated gradually became crystalline. Filtration and recrystallization of the material from 42% aqueous ethanol yielded 550 mg. (51%) of the pentaacetate IV, m.p. 116–117°, $[\alpha]^{16}D$ +28.6° (*c* 0.90, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.67. Found: C, 49.49; H, 5.75.

2,3,4,6-Tetra-O-acetyl-α-L-mannosyl Bromide (V).—To 8 ml. of acetic acid saturated with hydrogen bromide at 0° was added 2.0 g. (5.13 mmoles) of 1,2,3,4,6-penta-O-acetyl-β-L-mannose (IV), and the mixture was allowed to stand at room temperature for 3 hr. It was then delivered to a separatory funnel containing a small quantity of crushed ice, and 200 ml. of ether, previously cooled to 0°, was added. The ether solution was extracted rapidly in turn with 100 ml. of ice water, 100 ml. of saturated aqueous sodium bicarbonate (0°), and 100 ml. of ice water. After separating, the ether solution was dried over sodium sulfate and evaporated to dryness at 30° giving a sirup which was dissolved in 15 ml. of anhydrous ether, followed by the addition of 15 ml. of *n*-hexane. After standing in a refrigerator overnight, there was obtained 1.2 g. of pure V, m.p. 56–58°, $[\alpha]^{25}D$ −138.9° (*c* 0.80, $CHCl_3$). From the mother liquor an additional 0.7 g. was obtained, bringing the total yield of V to 1.7 g. (91%).

Anal. Calcd. for $C_{14}H_{19}O_9Br$: C, 40.88; H, 4.65; Br, 19.43. Found: C, 40.60; H, 4.61; Br, 19.26.

3β-(α-L-Mannopyranosyl)-5β,14β-dihydroxy-19-oxo-5β-card-20(22)-enolide (6'-Hydroxyconvallatoxin) (IX).—The coupling of strophanthidin (VI) with 2,3,4,6-tetra-O-acetyl-α-L-mannosyl bromide (V) and saponification of the reaction products according to the general procedure given gave 60 ml. of aqueous solution which was extracted in a manner identical with that described for the preparation of the D-mannoside VII, using the same battery of solvents. From the chloroform–ethanol (9:1 and 9:2) extracts there was obtained 240 mg. (33%) of crude material which, when recrystallized 4 times from ether–ethanol (5:95), gave 170 mg. (23%) of pure 6'-hydroxyconvallatoxin monohydrate (IX), m.p. 262–265°, $[\alpha]^{26}D$ +6.28° (*c* 0.42, 90% methanol), λ_{max}^{EtOH} 218 mμ (4.19). The cardenolide gave a positive Kedde test and when chromatographed on paper by an ascending technique employing 1-butanol saturated with water, had R_f 0.31.

Calcd. for [M] [strophanthidin (VI)] + [M] (methyl α-L-mannopyranoside)[14]: +17400° − 17850° = −450°. Calcd. for [M] [strophanthidin (VI)] + [M] (methyl β-L-mannopyranoside)[14]: +17400° + 16490° = +33890°. Found for [M] [strophanthidin-α-L-mannopyranoside (IX)]: +3667°. The glycosidic linkage in IX has therefore the α-configuration.

Anal. Calcd. for $C_{29}H_{42}O_{11}\cdot H_2O$: C, 59.60; H, 7.53. Found: C, 59.70; H, 7.54.

(12) F. Klages and R. Maurenbrecher, *Ann.*, **535**, 182 (1938).

(13) Calculated from the value of the corresponding methyl α-L-rhamnopyranoside, given in F. J. Bates and Associates, "Polarimetry, Saccharimetry, and the Sugars," U. S. Government Printing Office, Washington, D. C., 1942, p. 750.

(14) Calculated from the value of the corresponding methyl D-mannopyranoside given in ref. 14.

To remove any doubts concerning its composition, the cardenolide IX was recrystallized from absolute ether–methanol and was dried at 150° for 4 hr. (0.1 mm.), giving anhydrous material[15] melting at 265–269°.

Anal. Calcd. for $C_{29}H_{42}O_{11}$: C, 61.46; H, 7.47. Found: C, 61.72; H, 7.76.

Tetra-O-acetyl-6′-hydroxyconvallatoxin (IXa).—To a solution of 1 ml. of acetic anhydride in 1.2 ml. of pyridine was added 50 mg. (0.085 mmole) of 6′-hydroxyconvallatoxin monohydrate (IX). After standing for 24 hr. at 0°, the mixture was poured into ice water, and was extracted with chloroform–ether (1:3). The extract thus obtained was washed successively with 5% aqueous sulfuric acid, 5% aqueous sodium bicarbonate, and water. After drying over sodium sulfate, the extract was filtered and evaporated *in vacuo*, giving 35 mg. (56%) of the tetraacetate IXa as amorphous powder. The material was further purified by chromatography on silicic acid (Fisher Reagent Grade, activated) and from the chloroform–ether (1:1), methanol–chloroform–ether (1:49.5:49.5), and methanol–chloroform–ether (5:47.5:47.5) eluates there was obtained pure IXa as amorphous powder, m.p. 128–133°. For analytical purposes the material was dried for 5 hr. at 80° (0.1 mm.).

Anal. Calcd. for $C_{37}H_{50}O_{15}$: C, 60.50; H, 6.86. Found: C, 60.42; H, 7.17.

(15) The desolvation was attended with considerable difficulty. When the monohydrate IX was heated at lower temperatures, even for long periods of time, no desolvation took place. Drying at 150° for periods longer than that prescribed (6–8 hr., for example) brought about some decomposition as evidenced in the analytical results which were high with respect to carbon.

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New Synthesis and Structure Activity Relationship in the 17-Alkylated Progesterone Series

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A new synthesis for 17α-alkylated pregnane derivatives, by reductive alkylation of 16-dehydro-20-keto-pregnanes, is described. Structure–activity relationships in the 17α-alkylprogesterone series are presented and compared with that of known substances. The probable biological role of substituents capable of imparting oral activity to these substances is discussed. The ability of blocking metabolic transformations or inactivation following oral administration is considered a major factor for activity in this series of steroid hormone analogs.

Progesterone is the least polar of all steroids of biological sources.[1] Besides its importance in pregnancy, it is an intermediate in the biosynthetic pathway of corticoids, androgens and estrogens.[2] Perhaps for this reason of biological economy all hydroxylated progesterones[3] are less active than their precursor; probably polarity and metabolic factors are more important than stereochemical ones in destroying the "progestational" activity. Molecules with drastically different stereochemistry like retroprogesterone[7] and Ehrenstein's 14-iso-17-iso-19-norprogesterone[8] are known to be active, but introduction of a single hydroxyl function in position 11, for instance, of progesterone is sufficient to greatly reduce its activity.[9]

Metabolic studies with radioactive progesterone have shown[11] that the steroid is cleared from blood plasma at an extremely fast rate and that degradation of the side chain (as determined from analysis of labeled carbon dioxide in the expired air) plays an important role in the metabolic fate of the hormone.

Other metabolic "inactivations" include hydroxylation at position 16α,[12,13] 17α,[14] reduction of the 20-ketone and inversion of the acetyl side chain to the thermodynamically less stable α configuration.[15]

Protection of the side chain is therefore an essential prerequisite for oral activity or absence of side effects due to metabolites, and 17α substituents meet at least partly this requirement. Halogens,[16] alkyl groups,[17] and acyloxy groups[18] have been found to increase the oral activity of the molecule; of these however, 17-halogenated progesterones were disappointing in humans,[19] and 17α-acetoxyprogesterones, known to

(1) R. V. Short, in "Hormones in Blood," Academic Press, London and New York, 1961, p. 379 ff.

(2) J. K. Grant, *Brit. Med. Bull.*, **18**, 99 (1962).

(3) One notable exception is 17α-hydroxyprogesterone, devoid of activity in the rabbit and humans, but 60 times as potent as progesterone in the Rockland–Swiss mouse (Hooker–Forbes assay) even by systemic injection.[4,5] The fact that enzymes responsible for 17α-hydroxylation are missing from rat and mouse adrenal cortex[2] is perhaps not coincidental. For a critical evaluation of the test *cf.* ref. 6.

(4) H. A. Salhanick, E. G. Holstrom, and M. X. Zarrow, *J. Clin. Endocrin. Metab.*, **17**, 667 (1957).

(5) T. R. Forbes, "Brook Lodge Symposium on Progesterone," Brook Lodge Press, Augusta, Michigan, 1961, p. 71.

(6) H. Weifenbach, *Endokrinologie*, **40**, 13 (1960).

(7) A. Bompiani and E. Moneta, *Ann. Ostet. Ginecol.*, **84**, 607 (1962).

(8) M. Ehrenstein, G. W. Barber, and R. Hertz, *Endocrinology*, **60**, 681 (1957).

(9) The high activity of Δ^{11}-progesterone[10] is possibly due to impeded metabolic hydroxylation at position 11.

(10) Ch. Meystre, E. Tschopp, and A. Wettstein, *Helv. Chim. Acta*, **31**, 1463 (1948).

(11) E. J. Plotz, "Brook Lodge Symposium on Progesterone," Brook Lodge Press, Augusta, Michigan, 1961, p. 91.

(12) H. I. Calvin and S. Lieberman, *Biochemistry*, **1**, 639 (1962).

(13) J. Zander, J. Thijssen, and A. M. von Münstermann, *J. Clin. Endocrin. Metab.*, **22**, 891 (1962).

(14) B. Little and A. Shaw, *Acta Endocrin.*, **36**, 455 (1961).

(15) L. R. Axelrod and J. W. Goldzieher, *J. Clin. Endocrin. Metab.*, **20**, 238 (1960).

(16) C. I. Chappel, C. Revesz, and R. Gaudry, *Acta Endocrin.*, **35**, (Suppl. 51), 915 (1960).

(17) R. Deghenghi, Y. Lefebvre, P. Mitchell, P. F. Morand, and R. Gaudry, *Tetrahedron*, **19**, 289 (1963).

(18) J. C. Stucki and E. M. Glenn, "Brook Lodge Symposium on Progesterone," Brook Lodge Press, Augusta, Michigan, 1961, p. 25.

(19) D. J. Marshall, private communication.