droxide. After 1 hr. 8 ml. of methanol was added to the suspension and stirring was continued for 1.5 hr. The resulting solution was acidified, diluted with ice water, and extracted with ether. The ether solution was extracted with 10% aqueous sodium carbonate. The ethereal layer upon evaporation gave a semisolid (36 mg.) melting at 200–217° which was not further characterized. The aqueous portion on acidification and filtration yielded 325 mg. of crude Vb, m.p. 160–162.5°. Recrystallization from 50% aqueous methanol raised the melting point to 177–179° (reported[4] for a presumed imide XVIb, m.p. 180–182°). ν_{max} 3340 (OH), 2600 (OH of CO_2H), 2250 (C≡N), 1690 cm.$^{-1}$ (CO_2H).

Hydrolysis of 3β-hydroxy-16,17-seco-androstane-16-nitrile-17-oic Acid (Vb) in Aqueous Alkali.—A solution of 100 mg. of nitrile acid Vb in 10 ml. of 10% aqueous potassium hydroxide was heated under reflux for 10 hr. At the end of this heating period evolution of ammonia was noted. Acidification afforded 10 mg. of a solid, m.p. 208–210° (reported[4] for crude amide XIb, m.p. 210–214°). An infrared spectrum of the material indicated the presence of amide XIb (3320, 1640, 1600 cm.$^{-1}$) and diacid XVIIb (2600, 1710 cm.$^{-1}$). Recrystallization from methanol lowered the melting point to 190–192°.

Chromatography of the above product on silicic acid (Mallinckrodt A. R., 100 mesh, processed by stirring with water and drying at 110°) gave a small amount of impure diacid XVIIb, eluted with benzene, m.p. 227–237° (lit.,[4] m.p. 234–237°), and crude amide acid XIb, eluted with acetone–benzene (1:1), m.p. 209–214° dec. (lit.,[4] m.p. 210–214°). There was not enough material for further purification but the infrared spectra were consistent with the assignments as stated above.

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Partial Synthesis of Evomonoside[1]

W. WERNER ZORBACH,[2] GEORGE D. VALIAVEEDAN,[3] AND D. V. KASHELIKAR[4]

Department of Chemistry, Georgetown University, Washington, 7, D. C.

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Digitoxigenin was treated with 2,3,4-tri-*O*-benzoyl-α-L-rhamnosyl bromide to give an *O*-benzoylated glycoside which, after removal of the protecting groups, yielded digitoxigenin α-L-rhamnopyranoside, identical with the naturally occurring cardiac-active principle, evomonoside. Thus, the proof of structure of evomonoside has been completed. The α-D-rhamnopyranoside of digitoxigenin was made by similar methods and was found to have an infrared absorption spectrum nearly identical to that of evomonoside. Finally, digitoxigenin coupled readily with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate to give an acetylated glucosiduronate which could be isolated. Repeated attempts to saponify the latter resulted in failure.

In 1953, Tamm and Rosselet[5] elucidated the structure of evomonoside [3β-(α-L-rhamnopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (VI)], but they were unable to complete the proof of its structure by a partial synthesis. When they treated digitoxigenin [3β,14β-dihydroxy-5β-card-20(22)-enolide (V)] with 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl bromide in the presence of silver carbonate, elimination of the C-14 hydroxyl group of the genin (V) took place simultaneously with glycoside formation. Saponification of the *O*-acetylated intermediate gave, instead of the desired evomonoside (VI), 3β(α-L-rhamnopyranosyl)-5β-carda-14,20(22)-dienolide.

In an effort to resolve this problem we turned to a consideration of *O*-benzoylated halides as carbohydrate coupling intermediates inasmuch as the latter are considerably less reactive than the corresponding *O*-acetylglycosyl halides. The known 2,3,4-tri-*O*-benzoyl-α-L-rhamnosyl bromide (I)[6] was prepared easily from L-rhamnose but when I was treated with digitoxigenin (V) in the presence of silver carbonate under conditions essentially the same as described by Meystre and Miescher,[7] only 6% of evomonoside (VI) could be obtained after saponification of the reaction products.

Studies by Helferich and co-workers[8] have shown that mercuric cyanide may advantageously replace silver carbonate as an acid acceptor in the preparation of glycosides. Following Helferich's procedure except for substitution of 1,2-dichloroethane for the solvent nitromethane, we were able to couple the bromide I with the genin V; saponification of the reaction products *in toto* gave 44% of evomonoside (VI). Identity of the synthetic material with authentic naturally occurring evomonoside (VI)[9] was established on the basis of mixture melting point, paper chromatographic comparisons, and infrared spectra.

Natural cardenolides containing rhamnose as a

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(2) To whom all enquiries concerning this paper should be addressed.

(3) The preparation of 2,3,4-tri-*O*-benzoyl-α-D-rhamnosyl bromide which is described in this paper is taken from a dissertation submitted by G. D. Valiaveedan to the Graduate School of Georgetown University, June, 1960, in partial fulfillment of the M.S. degree.

(4) Visiting Scientist, Georgetown University, 1960-1961.

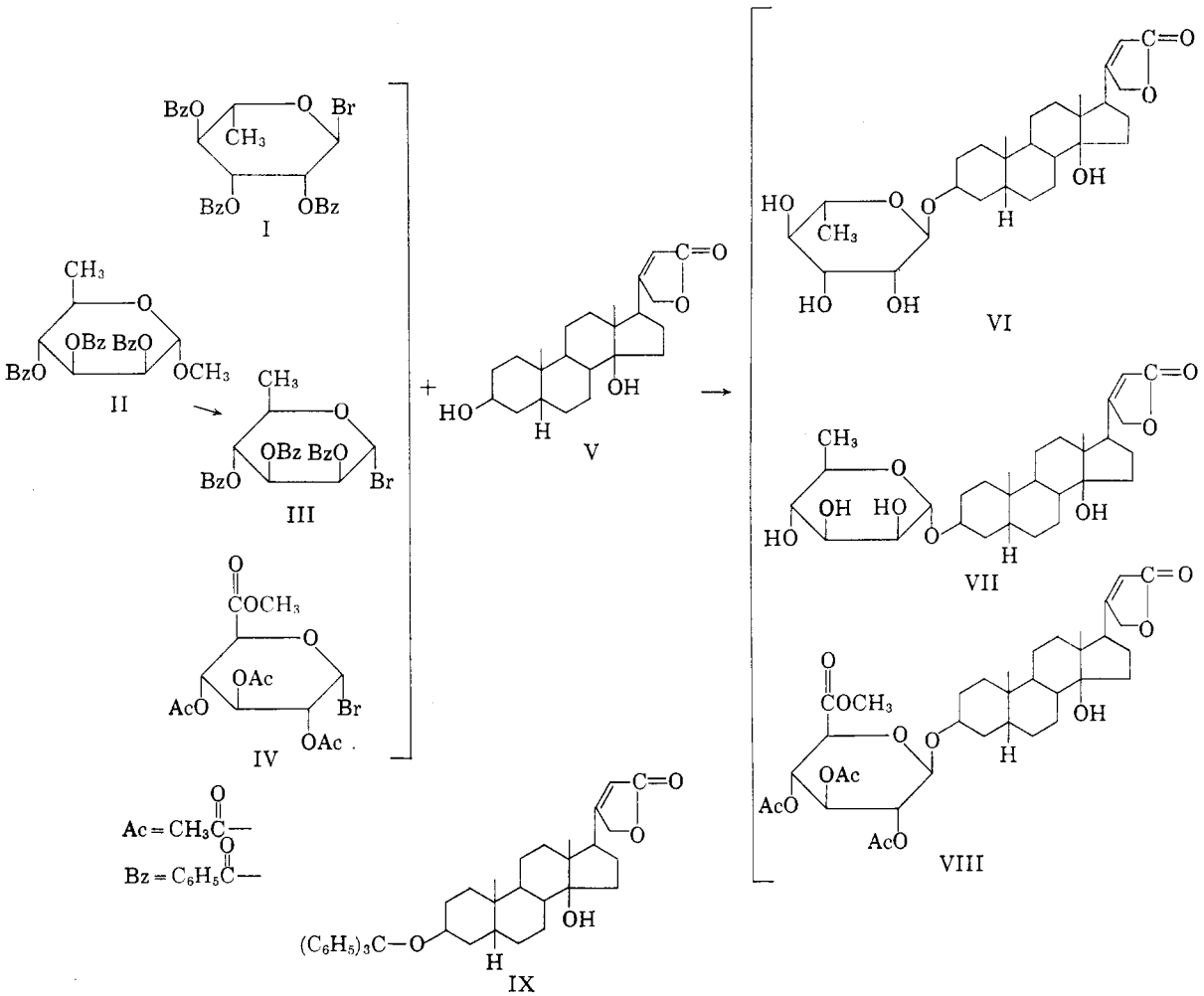
(5) Ch. Tamm and J. P. Rosselet, *Helv. Chim. Acta.*, **36**, 1309 (1953).

(6) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, *J. Am. Chem. Soc.*, **73**, 296 (1951).

(7) Ch. Meystre and K. Miescher, *Helv. Chim. Acta*, **27**, 231 (1944).

(8) B. Helferich and K. Weis, *Chem. Ber.*, **89**, 314 (1956); B. Helferich and R. Steinpreis, *Chem. Ber.*, **91**, 1794 (1958).

(9) The authors are grateful to Prof. T. Reichstein for supplying a sample of natural evomonoside.



carbohydrate component are formed only with the L-enantiomeric form of the sugar; cardenolides containing D-rhamnose are not known. Perhaps the most potent of all the natural cardenolides is convallatoxin (strophanthidin-α-L-rhamnopyranoside) and, in view of the vital role of the glycosidically bound sugar or sugars in enhancing or otherwise modifying the cardiac activity which resides in the genin, it was of interest to prepare some D-rhamnopyranosyl cardenolides in which all of the substituents on the lactol ring of the pyranoside would be reversed.

Two syntheses of the non-naturally occurring D-rhamnose are available.[10] The earlier of these, by Haskins, Hann, and Hudson, proceeds through the intermediate methyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannoside (II) which suggested a convenient route to an *O*-benzoylated rhamnosyl halide. Frequently, direct replacement by halogen of methoxyl in a methyl *O*-acylated glycopyranoside proceeds with difficulty and in a few cases not at all. Fortunately treatment of II with hydrogen bromide in acetic acid resulted in the slow replacement of the methoxyl group by bromide ion to give 2,3,4-tri-*O*-benzoyl-α-D-rhamnosyl bromide (III), corresponding with respect to physical constants to the bromide I, except that the sign of its rotation was reversed. Compounds I and III are, therefore, enantiomers.

Contrary to our expectations, the coupling of the D-rhamnosyl bromide III with digitoxigenin (V) in the presence of silver carbonate gave satisfactory yields. Without isolating the protected intermediate, the reaction products were carefully saponified to give 23% of 3β-(α-D-rhamnopyranosyl)-14β-hydroxy-5β-card-20(22)enolide (VII), the anomeric configuration of which was assigned by application of Klyne's rule of molecular rotational additivities.[11] The infrared spectrum of VII is of especial interest and, on first examination, appeared to be identical with the spectrum of evomonoside (VI), but closer scrutiny revealed a few differences which were barely discernable. Attention is invited to a consideration of the similarity between the spectra of the two anomeric monodigitoxosides of digitoxigenin[12] which differ only by

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

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

(12) W. W. Zorbach and T. A. Payne, *J. Am. Chem. Soc.*, **82**, 4979 (1960).

the configuration at the reducing carbon atoms of the bound sugars. Nevertheless, this difference would be expected to elicit significant changes in the vibrations of one anomer as compared to the other, inasmuch as the spatial orientation of the entire pyranoside ring with respect to the genin would be different.

Returning to the spectra of VI and VII it is reasonable to conclude that both pyranoside rings have equivalent conformations and, inasmuch as the anomeric configuration of the two are the same, then the structure of VI differs from that of VII only by a reversal of configuration of substituents of the bound sugar. It is well known that the infrared spectra of enantiomers are identical and on this basis it may be argued that compounds VI and VII should have nearly identical vibrational characteristics. This is predicated on the observation that in the steroid portion of both molecules, there are in the vicinity of the pyranoside ring no groups present which could give rise to a significantly different set of interactions for each of the glycosides VI and VII.

The new cardenolide VII showed a definite digitalis-like action and in ten cats the mean (geometric) lethal dose was found to be 0.6149 ± 0.0365 mg. kg.$^{-1}$.[13] A comparison of VII with evomonoside (VI) and with digitoxin (U.S.P. reference standard) is given in Table I.

TABLE I

Substance	LD(mg. kg.$^{-1}$)
Digitoxigenin-3-(α-D-rhamnopyranoside) (VII)	0.615
Digitoxigenin-3-(α-L-rhamnopyranoside) (evomonoside) (VI)	0.278
Digitoxin	0.325

The vital role of D-glucuronic acid in the body as a detoxifying agent led us to investigate the preparation of a D-glucosiduronic acid of digitoxigenin (V). When digitoxigenin (V) was treated with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (IV)[14] in the presence of silver oxide, the fully protected intermediate VIII was obtained and was easily isolable in crystalline form. Unfortunately compound VIII proved to be extremely resistant to saponification, and all attempts to remove the protecting groups in an effort to secure the free glucosiduronic acid resulted in impure returned starting material. Swiss workers independently have synthesized the *O*-acetylated glucuronate VIII and have had similar difficulties in attempts to proceed to the free acid.[15]

An alternate method of glycoside formation, described by Bredereck and co-workers,[16] was investigated for the preparation of VIII. Digitoxigenin (V) was converted to its 3-trityl ether IX. Treatment of IX with molar equivalents of IV and silver perchlorate in dry nitromethane failed, however, to give isolable quantities of VIII; consequently, the investigation of this latter procedure was discontinued.

Experimental

All melting points were determined using a Kofler hot stage. Paper chromatograms of the cardenolides were carried out as follows: A Whatman No. 1 paper was immersed for 15 sec. in 7.5% (v./v.) solution of formamide in acetone and after removing was dried in air for 15 min. Solutions of 1 mg. of cardenolide per 1 ml. of ethanol were prepared and 50 μl. of each were used for spotting. After equilibrating for 2 hr. the chromatograms were developed for 14 hr. by an ascending technique employing as the mobile phase tetrahydrofuran–isopropyl ether (2:3). After developing, the papers were removed and dried in a circulating oven at 80°. Spots were located by means of Kedde reagent.

3β-(α-L-Rhamnopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide(evomonoside) (VI).—To a solution of 374 mg. (1 mmole) of digitoxigenin (V) and 1078 mg. (2 mmoles) of 2,3,4-tri-*O*-benzoyl-α-L-rhamnosyl bromide (I) in 25 ml. of dry 1,2-dichloroethane was added 506 mg. (2 mmoles) of finely powdered mercuric cyanide. The solution was stirred magnetically for 24 hr. during which time dry nitrogen was bubbled slowly through the reaction mixture to prevent an accumulation of hydrogen cyanide, and losses of solvent through evaporation were restored by the periodic addition of dry 1,2-dichloroethane. The solution was filtered and the clear filtrate was evaporated *in vacuo* at 40°, leaving an amorphous residue which was extracted thoroughly with five 50-ml. portions of dry 1,2-dichloroethane. The combined dichloroethane extracts were treated with Celite 545, were filtered, and the clear filtrate was evaporated *in vacuo* at 40°. The resulting sirupy residue was dissolved in 250 ml. of methanol and this solution was added dropwise, under efficient stirring, to a solution of 2 g. of potassium bicarbonate in 90 ml. of water. The latter turbid solution became clear after stirring for 11 days at room temperature and it was then evaporated *in vacuo* at 40° to a volume of *ca.* 80 ml. The solution thus reduced in volume was extracted with three 100-ml. portions of ether all of which were combined, dried over magnesium sulfate, and filtered. Evaporation of the filtrate *in vacuo* gave 230 mg. (44%) of material (m.p. 213–225°) which, when recrystallized three times from ethanol–*n*-hexane (1:2.5), gave pure evomonoside (VI), m.p. 238–242°, undepressed when admixed with authentic material,[9] $[\alpha]^{18}D$ −28.7° (*c* 1.076, methanol) (Reichstein reported[17] $[\alpha]^{20}D$ −30.6 ± 2° in methanol), $\lambda_{max}^{CH_3OH}$ 218 mμ (4.21). Compound VI gave a positive Kedde and a negative tetranitromethane test. Synthetic VI and natural evomonoside[9] were chromatographed simultaneously on paper and both gave spots exactly coincident in position (R_f 0.385).

Anal. Calcd. for $C_{29}H_{44}O_8$: C, 66.90; H, 8.52; Found: C, 67.10; H, 8.37.

2,3,4-Tri-*O*-benzoyl-α-D-rhamnosyl Bromide (III).—A solution of 5.0 g. (10.2 mmoles) of methyl 2,3,4-tri-*O*-benzoyl-α-D-rhamnoside (II) in 20 ml. of glacial acetic acid at 45° was cooled to room temperature and 20 ml. of a solution of hydrogen bromide (*ca.* 35%) in acetic acid was added. After standing in a well stoppered flask for 24 hr., the reaction mixture was taken up in 150 ml. of dichloromethane previously cooled to 0° and the solution was

(13) The authors are much indebted to Dr. K. K. Chen, Eli Lilly and Co., Indianapolis, Ind., for carrying out this assay.

(14) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **111**, 347 (1935).

(15) Private communication from Dr. H. Lichti, Sandoz, Inc., Basel, Switzerland.

(16) H. Bredereck, A. Wagner, and G. Faber, *Angew. Chem.*, **69**, 438 (1957).

(17) H. Hauenstein, A. Hunger, and T. Reichstein, *Helv. Chim. Acta*, **36**, 87 (1953).

rapidly extracted in succession with 150 ml. of ice water, 150 ml. of saturated aqueous sodium bicarbonate (0°), and 150 ml. of ice water. After separating, the dichloromethane solution was dried over sodium sulfate and was evaporated *in vacuo* at 45°. Trituration of the resulting sirup with ether gave crude, crystalline material melting at 133–160°. The material was recrystallized from dry ether and by carefully working up the mother liquor there was obtained a total of 4.5 g. (82%) of 2,3,4-tri-*O*-benzoyl-α-D-rhamnosyl bromide (III), melting at 161–164°. Two additional crystallizations from dry ether gave pure III, m.p. 163–164°, $[\alpha]^{23}D$ −67.4° (*c* 1.65, chloroform).

Anal. Calcd. for $C_{27}H_{23}O_7Br$: C, 60.12; H, 4.30; Br, 14.81. Found: C, 60.41; H, 4.19; Br, 14.44.

3β-(α-D-Rhamnopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (VII).—To a magnetically stirred solution of 500 mg. (1.35 mmoles) of digitoxigenin (V) in 30 ml. of dry 1,2-dichloroethane contained in a 50-ml. flask equipped for distillation was added 1200 mg. of dry, freshly prepared silver carbonate. By heating the flask and contents in an oil bath, approximately one half of the solvent was caused to distill at a moderate rate. The flask was then fitted with a graduated dropping funnel which contained a solution of 1990 mg. (3.7 mmoles) of 2,3,4-tri-*O*-benzoyl-α-D-rhamnosyl bromide (III) in 200 ml. of dry 1,2-dichloroethane. The latter solution was added dropwise to the stirring mixture in the flask over a period of 3.5 hr., during which time the solvent from the reaction flask was permitted to distil over at an equal rate. After completion of the addition of the bromide III an additional 100 ml. of dry 1,2-dichloromethane was added over a period of 1.5 hr. with distillation maintained as in the foregoing. The insoluble silver salts were filtered and the filtrate was evaporated *in vacuo* at 40°. The sirupy residue was dissolved in 250 ml. of methanol and this solution was added dropwise to a stirring solution of 2.2 g. of potassium bicarbonate in 90 ml. of water. The latter turbid solution was stirred magnetically at room temperature for 15 days by which time it had become nearly clear. The solution was filtered with the aid of Celite 545 and was evaporated *in vacuo* at 38° to *ca.* 95 ml. The solution thus reduced in volume was extracted three times with 100-ml. portions of ether, all of which were combined and washed with three 50-ml. portions of water. After drying over magnesium sulfate, the ethereal extract was evaporated to dryness, giving a residue which, when recrystallized from 2-propanol, gave 37 mg. of product melting at 195–197°. The compound gave a negative Kedde test and was investigated no further.

The aqueous solution containing the saponified reaction products was extracted again with three 250-ml. portions of 1,2-dichloroethane–ethanol (9:1). After washing with 50 ml. of water, the combined extracts were dried over sodium sulfate and were evaporated *in vacuo* at 40°. The residue was dissolved in 10 ml. of hot 2-propanol and the solution was reduced in volume to 3 ml. by boiling. After cooling and filtering the separated material, there was obtained a total of 160 mg. (23%) of crystalline 3β-(α-D-rhamnopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (VII) which, when recrystallized five times from 2-propanol, gave pure VII, m.p. 253–254.5°, $[\alpha]^{23}D$ +53.4° (*c* 1.023, methanol) $\lambda_{max}^{CH_3OH}$ 218 mμ (4.23). The compound gave a positive Kedde and a negative tetranitromethane test, and when chromatographed on paper had R_f 0.377.

Calcd. for [M] (digitoxigenin (V) + methyl α-D-rhamnopyranoside[10a]): +71° +109° = +180°. Calcd. for [M] (digitoxigenin (V) + methyl β-D-rhamnopyranoside[18]): +71° −170° = −99°. Found for [M] (VII): +278°. The glycoside linkage in VII has therefore the α-configuration.[19]

Methyl (Digitoxigenin-3β-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate (VIII).—To a magnetically stirred solution of 748 mg. (2 mmoles) of digitoxigenin (V) in 25 ml. of dry 1,2-dichloroethane was added 2 g. of anhydrous magnesium sulfate and 2 g. of dry, freshly prepared silver oxide. To the stirring suspension was added 1589 mg. (4 mmoles) of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (IV) in 30 ml. of dry 1,2-dichloroethane over a period of 1 hr., and the resulting mixture was stirred for an additional 70 hr. at room temperature under the exclusion of light. The mixture was then filtered with the aid of Celite 545 and the clear filtrate was evaporated *in vacuo* at 40°. The sirupy residue was dissolved in 20 ml. of hot absolute ethanol and, after cooling, 576 mg. (42%) of crystalline material was obtained, losing solvent at 130° and melting at 222–225°. Two recrystallizations from absolute ethanol gave pure VIII, desolvating at 130–133° and melting at 225.5–228°, $[\alpha]^{20}D$ +2.1° (*c* 0.517, methanol). Calcd. for [M] [digitoxigenin (V)] + [M][methyl (methyl 2,3,4-tri-*O*-acetyl-α-D-glucosid)uronate]:[20a] +71° +605° = +676°. Calcd. for [M] (V) + [M][methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucosid)uronate]:[20b] +71° −101° = −30°. Found for [M] (VIII): +145°. The glucosidic linkage in VIII has therefore the β configuration. For combustion analysis, the sample was dried for 5 hr. at 150° under a reduced pressure of 0.1 mm.

Anal. Calcd. for $C_{35}H_{50}O_{13}$: C, 62.59; H, 7.30. Found: C, 62.34; H, 7.60.

3β-Triphenylmethoxy-14β-hydroxy-5β-card-20(22)-enolide (IX).—To a solution of 374 mg. (1 mmole) of digitoxigenin (V) in 1 ml. of dry pyridine was added 557 mg. (2 mmoles) of chlorotriphenylmethane. The flask containing the mixture was well stoppered and was heated at 75° for 18 hr. The contents of the flask were then transferred to a separatory funnel containing 175 ml. of ether and the resulting solution was extracted rapidly in succession with 60 ml. of 2 *N* sulfuric acid, 5% aqueous sodium bicarbonate, and water. After drying over magnesium sulfate the ethereal extract was filtered and was carefully evaporated to a volume of *ca.* 25 ml. by boiling. After standing for 1 hr. at room temperature, the contents of the flask were filtered giving 383 mg. of material melting at 260–275°. By concentrating the filtrate there was obtained an additional 100 mg. melting at 257–275°, bringing the total yield to 483 mg. (78%). One recrystallization from absolute ether gave pure IX, m.p. 273–281° dec., $[\alpha]^{20}D$ +13.6° (*c* 0.859, chloroform).

Anal. Calcd. for $C_{42}H_{48}O_4$: C, 81.78; H, 7.84. Found: C, 81.50; H, 7.91.

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(18) 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.

(19) The seemingly large discrepancy between the calculated and the observed molecular rotations of the α-D-rhamnoside VII might be reasonably accounted for in part by the fact that the observed rotations of the methyl α- and β-L-rhamnopyranosides were determined using aqueous solutions. Inasmuch as digitoxigenin (V) and the cardenolide VII are insoluble in water, methanolic solutions of the latter substances were used for their rotational measurements.

(20) (a) E. Hardegger and D. Spitz, *Helv. Chim. Acta*, **32**, 2165 (1949); (b) *Ibid.*, **33**, 337 (1950).