mesh Chromosorb W with 2% phosphoric acid) provided an estimate of the purity of the fatty acid esters, the extent of side reactions, and the partial depletion of benzophenone. Gas chromatograms indicated that the isomerization is not accompanied by significant side reactions. A Beckman IR-5A spectrophotometer was used for the quantitative measurements and the length of the sodium chloride cells was determined by counting interference fringes.

The methyl oleate used in this work was better than 99% pure and obtained from the Applied Science Laboratories, State College, Pa. In addition to chromatographic analysis, the purity of the fatty acid ester was estimated by the absence of absorption in the ultraviolet region. At 250 mμ, in isopropyl alcohol, the molar extinction coefficient was 4.5. Methyl elaidate was prepared by esterification of elaidic acid (Chemical Procurement Laboratories, Inc., College Point, N. Y.) with methanol and sulfuric acid. A low-temperature recrystallization in acetone of the prepared ester removed an impurity, which appeared to be eleostearic acid from its ultraviolet absorption. The purity of the elaidate sample was determined to be 99% by gas chromatography and the molar extinction coefficient at 250 mμ, in isopropyl alcohol, was determined to be 4.8.

Baker's analytical reagent grade benzene was used as solvent, and benzophenone (Matheson Coleman and Bell) was recrystallized from ethanol and hexane.

Methyl (Cortison-21-yl 2,3,4-tri-*O*-acetyl-β-D-glucosid)uronate[1]

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February 11, 1964

In an effort to secure C-21 2-deoxyglycosides of hydrocortisone (11β,17α,21-trihydroxy-4-pregnene-3,20-dione) as potential antiinflammatory agents, we investigated a glycosidation procedure described[3] by Bredereck and co-workers. When, however, 21-tritoxyhydrocortisone (11β,17α-dihydroxy-21-tritoxy-4-pregnene-3,-20-dione, **1**) was treated in the presence of silver perchlorate with various 2-deoxy acylglycosyl halides, the isolation of crystalline products was not realized.

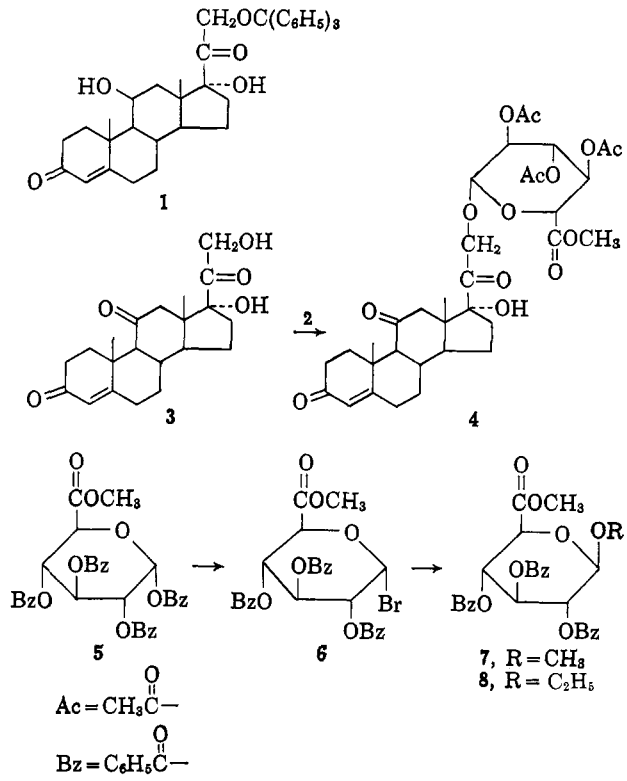
Also investigated was the preparation of some adrenocortical C-21 glucosiduronic acids as possible water-soluble derivatives. Whereas methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (**2**) coupled readily with cortexone (21-hydroxy-4-pregnene-3,20-dione) to give the desired glucosiduronate in good yield,[4] treatment of hydrocortisone with **2** under identical conditions failed to give crystalline material, even after chromatography of the reaction products on silicic acid.

We next turned our attention to the corresponding methylated and fully acetylated C-21 glucosiduronic acid (**4**) prepared from cortisone (**3**) and previously reported by Wotiz and co-workers.[5] Whereas these workers were able to isolate the crystalline intermediate directly from the reaction mixture, it was necessary for us to chromatograph the material on silicic acid. The crystalline material thus obtained had properties (melting point, rotation) which differed from the data reported by Wotiz for the coupling product (**4**). Also, combustion analysis for our compound (**4**) gave values for carbon and hydrogen closely fitting $C_{34}H_{44}O_{14}$ (the correct molecular formula), while Wotiz' values more nearly correspond to the formula $C_{34}H_{46}O_{14}$ which is the one assigned erroneously to **4** in his paper.

In the absence of further published information, we infer that Wotiz' compound may have been impure (in view of the sharp melting point reported this does not seem likely) or that it possibly represents an incorrectly named coupling product of **2** with some dihydro derivative or cortisone. The synthesis of **4** reported herein represents, then, the first successful coupling of cortisone (**3**) with D-glucuronic acid.

Saponification of **4** yielded amorphous material which could not be crystallized. The material was readily soluble in water giving a solution which was acidic to litmus. It was homogeneous as disclosed by papergrams and, when hydrolyzed by means of β-D-glucuronidase and again chromatographed on paper, showed two spots, exactly coincident in position with cortisone (**3**) and D-glucuronic acid, respectively.

In a further attempt to secure a C-21 glucosiduronic acid from hydrocortisone, we investigated benzoylated derivatives of D-glucuronic acid owing to the superior crystallizing properties of carbohydrate benzoates. Methyl D-glucuronate[6] was readily converted to the tetrabenzoate (**5**), which is provisionally assigned to the α-anomeric configuration because of its strongly positive specific rotation (+125.2°). Treatment of **5** with hydrogen bromide–acetic acid solution gave the expected methyl 2,3,4-tri-*O*-benzoyl-1-bromo-1-deoxy-α-D-glucuronate (**6**), which underwent methanolysis to



(1) This work was supported in part by U. S. Public Health Service Grant AM02764.

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give a methyl (methyl 2,3,4-tri-*O*-benzoyl-D-glucosid)-uronate (**7**). Because 1,2-*cis* halides undergo displacement with inversion at C-1, **7** has most likely the β-anomeric configuration.

Several experiments were carried out in attempts to couple hydrocortisone with the bromide (**6**) in the presence of mercuric cyanide but, in each instance, substantial amounts of unreacted hydrocortisone could be recovered. In one case, during a work-up of the reaction mixture using ethanol, methyl (ethyl 2,3,4-tri-*O*-benzoyl-D-glucosid)uronate (**8**) was isolated, indicating that throughout the experiment the bromide (**6**) remained unaffected. Because **8** also is levorotatory, it most probably has the β-anomeric configuration.

Experimental

All melting points were determined using a Kofler hot stage.

11β,17α-Dihydroxy-21-tritoxy-4-pregnene-3,20-dione (1).—To a solution of 1086 mg. (3 mmoles) of hydrocortisone in 5 ml. of anhydrous pyridine was added 921 mg. (3.3 mmoles) of trityl chloride. Under exclusion of moisture the solution was heated on a steam bath for 4 hr. and was then dissolved in 300 ml. of ether. After washing successively with 200 ml. of 1 *N* sulfuric acid, 200 ml. of 2% aqueous sodium bicarbonate, and 200 ml. of water, the ether layer was dried over sodium sulfate and was evaporated *in vacuo* at 40° to a sirupy residue. The residue was triturated with 5 ml. of absolute ethanol and, after standing overnight in a refrigerator, there was obtained 1180 mg. (65%) of pure **1**, m.p. 197.5–201° dec., $[\alpha]^{19}D$ +77.1° (*c* 1.188, $CHCl_3$). From the mother liquors an additional 110 mg. of pure **1** was obtained, bringing the total yield to 71%.

Anal. Calcd. for $C_{40}H_{44}O_5$: C, 79.43; H, 7.34. Found: C, 79.35; H, 7.51.

Methyl (17α,21-Dihydroxy-4-pregnene-3,11,20-trion-21-yl 2,-3,4-tri-*O*-acetyl-β-D-glucosid)uronate (4).—The coupling of 1500 mg. (3.8 mmoles) of the bromide (**2**) in 70 ml. of anhydrous benzene with 540 mg. (1.5 mmoles) of cortisone (**3**) in the presence of 1450 mg. (5.25 mmoles) of freshly prepared silver carbonate was carried out by an azeotropic distillation technique, directions for which are given[7] by Reichstein and co-workers. After completion of the reaction, the silver salts were filtered and the filtrate was evaporated to dryness giving 1.5 g. of sirupy material which was placed on a column (3 × 48 cm.) of 75 g. of Fisher reagent grade silicic acid. The material which was eluted by dichloromethane methanol (95:5) was collected, and the solution was decolorized with Darco G-60. After filtration and evaporation, the clear sirup was redissolved in methanol and water was added to incipient turbidity. After standing overnight in a refrigerator, the separated crystals were filtered giving 220 mg. (32%) of material melting at 110–118°. Repeated recrystallization from water–methanol gave pure **4**, m.p. 127–129°, $[\alpha]^{18}D$ +103.1° (*c* 0.78, $CHCl_3$), λ_{max}^{MeOH} 239 mμ (log ε 4.1).

Anal. Calcd. for $C_{34}H_{44}O_{14}$: C, 60.34; H, 6.55. Found: C, 60.25; H, 6.82.

Saponification of 4 and Enzymatic Hydrolysis of the Deacetylated Acid.—To a solution of 10 mg. of the protected glucosiduronate (**4**) in 10 ml. of absolute methanol was added 1 ml. of 0.5 *N* sodium methoxide. After standing overnight at room temperature, the solution was made neutral by the addition of acetic acid and was then diluted with an equal volume of water. After treating for 0.5 hr. with 500 mg. of Amberlite MB-1 ion-exchange resin, the solution was filtered and was evaporated to dryness *in vacuo* at 40°. The amorphous residue gave an acid reaction with litmus and did not reduce ammoniacal silver oxide. It was next dissolved in 50 ml. of acetate buffer (pH 5.2) and 250 mg. of β-D-glucuronidase (Nutritional Biochemicals Corp., 60,000–70,000 units/g.) was added. After incubating for 24 hr. at 37°, the solution was cooled and extracted with three 100-ml. portions of ether. After drying over magnesium sulfate, the extract was evaporated and the residue was dissolved in 2 ml. of methanol for paper partition chromatography by an ascending method employing 1-butanol–acetic acid–water (4:1:5). This system appears to be satisfactory for both sugars and corticoids and, when the hydrolysate was chromatographed, there was a good resolution of the material giving two spots, exactly coincident in position with D-glucuronic acid and cortisone (**1**), respectively. The spots were detected using ammoniacal silver nitrate with subsequent drying at 110° for 3 min.

Methyl 1,2,3,4-Tetra-*O*-benzyl-α-D-glucuronate (5).—A solution of 19.6 g. (0.094 mole) of sirupy methyl glucuronate[6] in 75 ml. of pyridine was cooled to −10° and 50 ml. of benzoyl chloride (0.425 mole) was added dropwise with stirring. After stirring for an additional 0.5 hr., the mixture was set aside in a refrigerator for 4 days. It was then allowed to warm to room temperature and the excess benzoyl chloride was carefully neutralized by the addition of saturated aqueous sodium bicarbonate. The mixture was added rapidly to 2 l. of ice–water and was set aside in a refrigerator overnight. The gummy mass which separated was dissolved in 300 ml. of chloroform and the solution was washed with 2% aqueous sodium bicarbonate and with water. After drying over sodium sulfate, the extract was boiled down to 100 ml. and was treated with Darco G-60. After filtering, the solution was evaporated to dryness and the residue was redissolved in 300 ml. of ethanol. On the following day the separated material was filtered and was repeatedly crystallized from acetone–water, giving pure **5**, m.p. 183–184°, $[\alpha]^{25}D$ +125.2° (*c* 1.232, $CHCl_3$). Work-up of the mother liquors gave additional material, 30.0 g. (48%) *in toto*, of the glucuronate (**5**).

Methyl 2,3,4-Tri-*O*-benzoyl-1-bromo-1-deoxy-α-D-glucuronate (6).—To 50 ml. of a 32% hydrogen bromide–acetic acid solution was added 5.0 g. (8 mmoles) of finely powdered methyl 1,2,3,4-tetra-*O*-benzoyl-α-D-glucuronate (**5**), and the mixture was stirred under the exclusion of moisture for 4 hr. After standing at room temperature for 15 hr., the solution was poured into cold ether and was washed successively with 250 ml. of cold 2% aqueous sodium bicarbonate, and with 250 ml. of ice–water. The ether layer was dried over sodium sulfate and filtered, and then evaporated to dryness at 30°. The residue was redissolved in anhydrous benzene and dry pentane was added to incipient turbidity. After standing in a refrigerator overnight, the separated material was collected by filtration and, after three recrystallizations, there was obtained 2.9 g. (62%) of pure bromide (**6**), m.p. 146–148°, $[\alpha]^{18}D$ +114° (*c* 1.31, $CHCl_3$).

Anal. Calcd. for $C_{28}H_{23}BrO_9$: C, 57.63; H, 3.97; Br, 13.70. Found: C, 56.97; H, 4.20; Br, 13.62.

Methyl (Methyl 2,3,4-tri-*O*-benzoyl-β-D-glucosid)uronate (7).—To 150 mg. (0.26 mole) of the bromide **5** was added 10 ml. of anhydrous methanol and the solution was allowed to stand at room temperature for 16 hr. The solution was evaporated to dryness *in vacuo* at room temperature. The solid residue was recrystallized twice from methanol giving 93 mg. (70%) of pure **7**, m.p. 153–154°, $[\alpha]^{19}D$ −3.7° (*c* 0.822, methanol).

Anal. Calcd. for $C_{29}H_{26}O_{10}$: C, 65.16; H, 4.90. Found: C, 64.85; H, 5.13.

Isolation of Methyl (Ethyl 2,3,4-Tri-*O*-benzoyl-β-D-glucosid)-uronate (8) from the Attempted Coupling of the Bromide 6 with Hydrocortisone.—A solution of 362 mg. (1 mmole) of hydrocortisone in 15 ml. of absolute dioxane was treated with 583 mg. of **6** and 253 mg. (1 mmole) of mercuric cyanide according to directions given in a previous paper.[8] After completion of the reaction, the solvent was evaporated *in vacuo* and the resulting sirupy residue was redissolved in 5 ml. of absolute ethanol. After standing overnight, the separated material, which melted at 120–135°, was recrystallized three times from absolute ethanol giving 155 mg. of pure **8**, m.p. 150–152°, $[\alpha]^{19}D$ −5.6° (*c* 0.682, $CHCl_3$).

Anal. Calcd. for $C_{30}H_{28}O_{10}$: C, 65.69; H, 5.14. Found: C, 65.71; H, 5.07.

Acknowledgment.—The authors are indebted to Miss Paula M. Parisius, Microanalytical Laboratory, NIAMD, National Institutes of Health, Bethesda, Maryland, under the direction of Mr. H. G. McCann, for the elemental analyses.

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