THE JOURNAL OF Organic Chemistry®

VOLUME 36, NUMBER 7

APRIL 9, 1971

Steroid Conjugates. VI.[1a] An Improved Koenigs–Knorr Synthesis of Aryl Glucuronides Using Cadmium Carbonate, a New and Effective Catalyst[1b]

R. B. CONROW AND SEYMOUR BERNSTEIN*

Organic Chemical Research Section, Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965

Received September 1, 1970

The 3-β-D-glucuronide triacetate methyl esters of estrone, 17β-estradiol, estriol, equilin, and equilenin and the 3-β-D-glucoside tetraacetate of estrone were obtained in yields of 46–71% by direct crystallization from a Koenigs–Knorr reaction using a glycosyl halide and the novel catalyst, cadmium carbonate. This represents an approximately tenfold improvement in yield over previously reported methods. Evidence was obtained which suggests that the actual catalyst in these reactions is the resulting cadmium halide. Among the identified by-products were small amounts of the corresponding α anomers and the steroidal 3-acetates. A 4-*C*-glucuronosyl derivative of equilenin was also obtained in 14% yield. The products were deblocked by standard methods to give the corresponding glucuronides and glucoside.

The importance of glycoside synthesis in many areas of natural product chemistry is well documented.[2] Recently, steroid conjugates,[3] consisting primarily of sulfates and glucuronides, have attracted increasing attention. This stems largely from the growing awareness that their role in the body is not merely one of detoxification.[4] As a result, improved methods for preparing these compounds have assumed greater importance.

Generally, steroidal alicyclic glucuronides are reasonably accessible by present methods.[5] This, however, is not true with steroidal aryl glucuronides.[6,7] For example, reported[6a-c] yields of methyl [17-oxoestra-1,3,5(10)-trien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid]uronate (**4**) (henceforth abbreviated, estrone-3-β-D-glucuronide triacetate methyl ester) using silver carbonate in the standard Koenigs–Knorr reaction have not exceeded approximately 7%. Consequently, the isolation of product from such low yield reactions often necessitates tedious crystallization and countercurrent or chromatographic procedures. In connection with our investigation of the biological function of steroid conjugates, a more convenient method for obtaining these compounds was required. Toward this end, an investigation of the catalytic effect of various metals,[8] mainly as their carbonates or oxides, on the glucuronidation of estrone was undertaken. Next in

(1) (a) Part V: J. P. Joseph, J. P. Dusza, E. W. Cantrall, and S. Bernstein, *Steroids*, **14**, 591 (1969); (b) S. Bernstein, presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.

(2) For a recent, extensive review of glycosides, see (a) L. Hough and A. C. Richardson in "Rodd's Chemistry of Carbon Compounds," Vol. I[F], S. Coffey, Ed., Elsevier, Amsterdam, 1967, p 320. For additional reviews relating to the Koenigs–Knorr Synthesis, see (b) C. A. Marsh in "Glucuronic Acid," G. J. Dutton, Ed., Academic Press, New York, and London, 1966, p 62; (c) J. Conchie, G. A. Levvy, and C. A. Marsh, *Advan. Carbohyd. Chem.*, **12**, 157 (1957); (d) R. U. Lemieux, *ibid.*, **9**, 1 (1954); (e) W. W. Zorbach and K. V. Bhat, *ibid.*, **21**, 273 (1966); and (f) W. L. Evans, D. D. Reynolds, and E. A. Tally, *ibid.*, **6**, 41 (1951).

(3) For extensive references and reviews pertaining to steroid glucuronide conjugates, see (a) S. Bernstein, E. W. Cantrall, J. P. Dusza, and J. P. Joseph, "Steroid Conjugates, a Bibliography," Chemical Abstracts Service, American Chemical Society, 1966; (b) H. E. Hadd and R. T. Blickenstaff, "Conjugates of Steroid Hormones," Academic Press, New York and London, 1969; (c) S. Bernstein and S. Solomon, Ed., "Chemical and Biological Aspects of Steroid Conjugates," Springer-Verlag, New York, N. Y., 1970, in press; (d) S. Bernstein, J. P. Dusza, and J. P. Joseph, "Physical Properties of Steroid Conjugates," Springer-Verlag, New York, N. Y., 1968; and e) R. Hähnel and N. bin Muslim, *Chromatogr. Rev.*, **11** (3), 215 (1969).

(4) Reference 3b, p 293, and references therein.

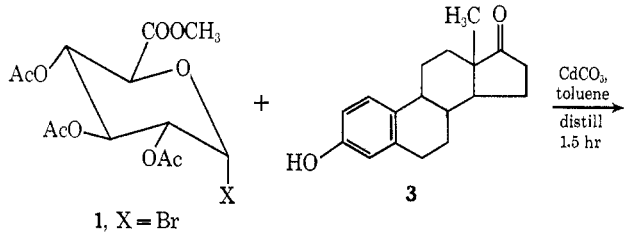
(5) For some examples of the preparation of steroid alicyclic glucuronides in good yield, see (a) J. J. Schneider and N. S. Bhacca, *J. Org. Chem.*, **34**, 1990 (1969); (b) J. F. Becker, *Biochim. Biophys. Acta*, **100**, 574 (1965); (c) V. R. Mattox, J. E. Goodrich, and W. D. Vrieze, *Biochemistry*, **8**, 1188 (1969); (d) Ch. Meystre and K. Miescher, *Helv. Chim. Acta*, **27**, 231 (1944).

(6) (a) E. Schapiro, *Biochem. J.*, **33**, 385 (1939); (b) J. S. Elce, J. G. D. Carpenter, and A. E. Kellie, *J. Chem. Soc.*, 542 (1967); (c) H. H. Wotiz, E. Smakula, N. N. Lichtin, and J. H. Leftin, *J. Amer. Chem. Soc.*, **81**, 1704 (1959); (d) T. Nambara and K. Imai, *Chem. Pharm. Bull.*, **15**, 1232 (1967).

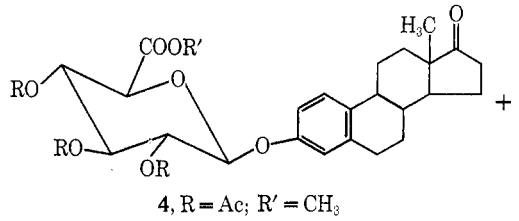
(7) A. Hagedorn, F. Johannessohn, E. Rabald, and H. E. Voss, *Z. Physiol. Chem.*, **264**, 23 (1940) [*Chem. Abstr.*, **34**, 4783[2] (1940)], report the preparation of estrone-3-β-glucoside Ac4 (**7**) in 63% yield from acetobromoglucose using quinoline–Ag_2CO_3 as condensing agent. Other workers [*e.g.*, (b) C. A. Marsh and L. M. Reid, *Biochim. Biophys. Acta*, **97**, 597 (1965); (c) F. G. Muhtadi and M. J. R. Moss, *Tetrahedron Lett.*, 3751 (1969); and (d) H. Tanino, S. Inoue, K. Nishikawa, and Y. Hirata, *Tetrahedron*, **25**, 3033 (1969)], have also found the combination of Ag_2CO_3 or Ag_2O with quinoline useful for the preparation of various aromatic glycosides. However, in our hands the glucuronidation of estrone by this method gave a thick dark mixture from which product could not be crystallized directly. Purification of a sample by tlc (system A) gave **4** in 23% yield.

(8) Helferich and coworkers investigated a variety of materials including the oxides of zinc, cadmium, and mercury as glycosidation catalysts, but mainly for primary alcohols: (a) B. Helferich and K. F. Wedemeyer, *Justus Liebigs Ann. Chem.*, **563**, 139 (1949) [*Chem. Abstr.*, **43**, 7430*g* (1949)]; (b) B. Helferich and K. F. Wedemeyer, *Chem. Ber.*, **83**, 538 (1950) [*Chem. Abstr.*, **45**, 3336*b* (1951)]; (c) B. Helferich and A. Berger, *Chem. Ber.*, **90**, 2492 (1957) [*Chem. Abstr.*, **52**, 16224*c* (1958)]. These workers found that $Hg(CN)_2$ was a particularly effective catalyst and it has since proved of value, expecially where Ag_2CO_3 or Ag_2O gave poor results. See ref 2e, p 278; 2c, p 166; and 2a, p 23.

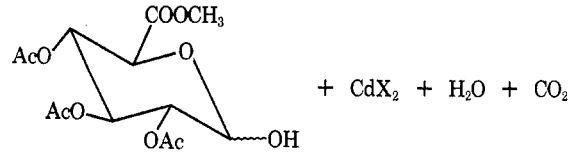
importance to silver carbonate and oxide as glycosidation catalysts are various salts of mercury, *e.g.*, $Hg(CN)_2$[8] and $HgO–HgBr_2$.[9] While use of the HgO catalyst system with methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyran)uronate (**1**)[10] (henceforth referred to as bromo sugar **1**) in refluxing toluene (procedure A) gave an improved yield (25%) of estrone-3-β-D-glucuronide triacetate methyl ester, the product was contaminated with organomercury complexes which were difficult to remove. The next element investigated was cadmium[8] inasmuch as this metal is in the same periodic group as mercury. The glucuronidation of estrone in the presence of $CdCO_3$ using procedure A afforded a 54% yield of the glucuronide **4**. Moreover, tlc indicated that the mixture was relatively uncom-



1, X = Br
2, X = Cl
3



4, R = Ac; R' = CH_3
5, R = H; R' = Na



6

plex, containing chiefly unreacted estrone (28%) in addition to the desired product. Further evidence for the catalytic superiority of cadmium carbonate under these conditions (procedure A) was exemplified by the results obtained with the following compounds: $ZnCO_3$, CdO,[8] CdS, $CoCO_3$, $NiCO_3$, $PbCO_3$,[11] $CuCO_3 \cdot Cu(OH)_2$, and NaOAc. Only the first three compounds gave any product, the yields being approximately 19, 38, and 20%, respectively. With $ZnCO_3$, a dark brown gum precipitated halfway through the reaction, due probably to decomposition of the bromo sugar **1**. It was not surprising that CdO and CdS gave some product since it was reasoned that, as with $CdCO_3$, the resulting $CdBr_2$ was probably the effective catalyst[12] in these reactions. This aspect will be discussed further in conjunction with other factors affecting the reaction.

(9) L. R. Schroeder and J. W. Green, *J. Chem. Soc. C*, 530 (1966).

(10) G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Amer. Chem. Soc.*, **77**, 3310 (1955).

(11) Lead carbonate has been used as a catalyst for the preparation of ortho esters: N. K. Kochetkov, A. J. Khorlin, and A. F. Bachkov, *Tetrahedron Lett.*, 289 (1964).

(12) In glycosidations using mercuric compounds, such as $Hg(CN)_2$ and HgO, the resulting halide is considered to be the active catalyst. See, for example, ref 9.

The initial results obtained with $CdCO_3$ were very encouraging and suggested that a proper selection of reaction conditions would result in complete reaction of the starting steroid. This was desirable not only to obtain a good yield of product but also to facilitate its isolation by direct crystallization from the crude mixture. Efforts in this direction showed that continuous distillation[13] of toluene from the mixture was more effective in bringing the reaction to completion than successive increases in the amount of bromo sugar **1**. A limited investigation of other reaction variables determined that complete reaction of the estrone was achieved when 2 equiv of bromo sugar were added dropwise, over 1 hr, to a mixture of the steroid and $CdCO_3$ in distilling toluene followed by an additional 0.5-hr reaction time. At this stage the organic soluble components of the mixture were predominately product and methyl (2,3,4-tri-*O*-acetyl-D-glucopyran)uronate (**6**).[14] Since the latter compound is water soluble, this allowed an initial purification of the product by dissolving it in dimethylformamide (or, better, acetone) and pouring the solution into water. The desired glucuronide **4** was precipitated in sufficient purity that three crystallizations from methylene chloride–ethanol provided pure material in an isolated yield of 71%. That this method is generally applicable to the preparation of other steroidal phenolic glycosides in good yield is demonstrated by the results in Table I.[15]

TABLE I

PREPARATION OF STEROIDAL PHENOLIC GLYCOSIDES *via* A $CdCO_3$ MEDIATED KOENIGS–KNORR REACTION

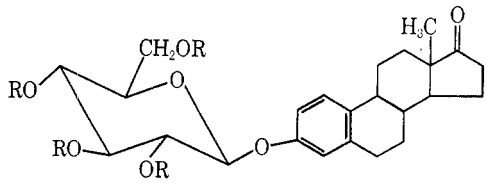
Products[a]	Compd no.	Yield, %[b]		Color of reaction
Estrone-3-β-GAc_3Me[c]	4	71.0		Pink
Estrone-3-β-$GlAc_4$[d]	7	61.0	67.5	Pink
Estrone-3-α-$GlAc_4$	9	6.5[e]		
Estradiol-17β-formate-3-β-GAc_3Me	10	71.0		Pale tan
Estriol-16α,17β-diformate-3-β-GAc_3Me	12	65.0		Pale tan
Equilin-3-β-GAc_3Me	14	68.0		Pink
Equilenin-3-β-GAc_3Me	16	46.0	62.0	Pink changing to pale tan
Equilenin-3-α-GAc_3Me	18	2.0[e]		
Equilenin-4-ξ-glucuronosyl Ac_3Me	19	14.0[f]		

[a] All products are new compounds except **4** and **7**. [b] Actual yield of product isolated by crystallization, unless otherwise indicated. [c] Stands for estrone-3-β-D-glucuronide triacetate methyl ester. [d] Stands for estrone-3-β-D-glucoside tetraacetate. [e] Isolated by chromatography. [f] Isolated by crystallization and chromatography.

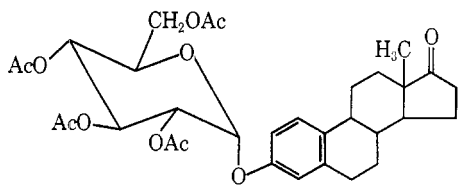
(13) This presumably removes water formed during the reaction and is the basis of the Meystre–Miescher[6d] modification of the Koenigs–Knorr reaction. Other workers have also noted that anhydrous conditions had a beneficial effect on yield. See, for example, ref 6b.

(14) In a similar run, a polar non-uv-absorbing band was isolated as a glass by preparative tlc. Although this material was still somewhat impure, its spectral properties (ir, nmr, and rotation) indicated that it was an anomeric mixture of **6** when compared to an authentic sample of the α anomer of **6** prepared by the method of N. Pravdić and D. Keglević, *J. Chem. Soc.*, 4633 (1964).

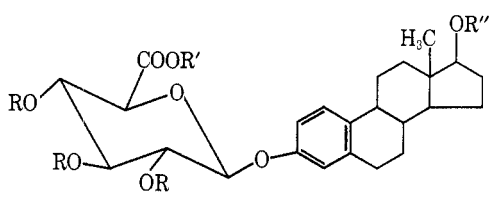
(15) In the limited number of examples tried, the method also gives good yields of steroidal alicyclic glucuronides, except where easily eliminated hydroxyls were involved, as in androsterone, digitoxigenin, and 17α-estradiol. Also, the formation of by-products, such as α anomers, tends to be greater than in the aromatic series: R. B. Conrow and S. Bernstein, unpublished results. Cadmium carbonate has also found use in the preparation of the anomeric *N*-acetylglucosaminides of 17α- and 17β-estradiol: J. P. Joseph, J. P. Dusza and S. Bernstein, Steroid Conjugates VII, submitted for publication in *Biochemistry*.



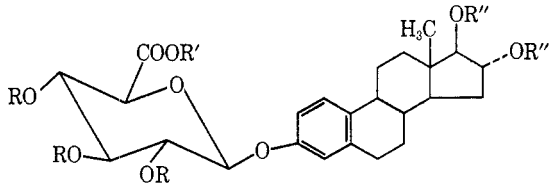
7, R = Ac
8, R = H



9



10, R = Ac; R′ = CH_3; R″ = CHO
11, R = R″ = H; R′ = Na



12, R = Ac; R′ = CH_3; R″ = CHO
13, R = R″ = H; R′ = Na

An interesting feature of the reaction is the development of color on the surface of the cadmium carbonate. This occurs with most of the substrates and in some cases is quite vivid, as indicated in Table I. This aspect will also be discussed further.

The structure of the acetylated β-D-glucuronides and glucosides were fully supported by elemental analysis and spectral studies,[16] including the mass spectrum.[17] Although isolation of all products formed in these reactions was not attempted, some of the more accessible steroid-containing by-products were investigated. In all of the reactions, a weakly polar, uv-absorbing product was observed. In the glucuronidation of estrone and equilenin this was identified as the corresponding steroid 3-acetate, obtained in a yield of approximately 2%. In the preparation of estrone-3-β-D-glucoside tetraacetate (**7**)[7a] and equilenin-3-β-D-glucuronide triacetate methyl ester (**16**), the corresponding α anomers[18] **9** and **18** were isolated in yields of 6.5 and 2%, respectively. It is likely that the other reaction mixtures also contained some of the α anomer, but these were either not evident by tlc or could not be isolated in sufficient purity for a positive identification. Initial evidence for the structure of the α anomers was provided by their infrared spectrum,[19] which showed distinct differences in the glycosidic bond region at 1000–1110 cm^{-1}, compared to the β anomer. Thus, the β anomer contains absorption in this region, as a peak or shoulder, which is absent in the α anomer. The net effect is to make the glycosidic bond–ester complex between 1010 and 1110 cm^{-1} appear sharper and somewhat more intense in the α anomer than in the β. The large difference in optical rotations[20] also suggested anomeric pairs. The most conclusive evidence, however, was provided by the nmr spectra which indicated an equatorial–axial relationship ($J_{1',2'}$ = 3.5 Hz)[21] for the C-1,2 sugar protons of the α anomers. It is feasible that the α anomers could be derived from the β as a result of the catalytic effect[22] of $CdBr_2$, or any free hydrogen bromide, formed in the reaction. A most interesting by-product, obtained in significant yield (14%) from the glucuronidation of equilenin, was the *C*-glycosyl compound[23] 4-ξ-glucuronosyl triacetate methyl ester, **19**. Its ir spectrum was similar to that of the glucuronide **16** except that it appeared to contain a hydroxyl group and showed differences in the glycosidic bond region.[19] The hydroxyl was confirmed and shown to be phenolic by the uv spectrum which evidenced a bathochromic shift on basification.[24] The failure to detect any equilenin on strong acid hydrolysis[23] of **19** (1:1 2 *N* HCl–EtOH, 4-hr reflux) decreased the possibility that it could have an *O*-glucuronide, ortho ester, or acetal type structure. Moreover, in the mass spectrum[25] of **19** the most abundant ions were those in which the sugar moiety was retained, whereas in the glucuronide **16** the most abundant ions were derived from the eliminated sugar moiety. These

(16) See, ref 2a, p 125, for a review of the structural determination of monosaccharides by physical methods.

(17) A majority of the abundant fragments in the mass spectrum of the glucuronide triacetate methyl esters could be interpreted as arising from loss of OMe, COOMe, acetic acid, and ketene fragments, and cleavage of the glucuronosidic bond. In the glucoside tetraacetate derivatives, the major fragments were associated with loss of OAc, CH_2OAc, acetic acid, and ketene together with cleavage of the glucosidic bond.[2] The fragments are listed (see Experimental Section) in order of decreasing abundance. The first series of numbers represents abundant fragments and the second series represents less abundant fragments in the high mass range.

(18) The formation of α anomers of simple glycosides has been reported when mercuric salts have been used in conjunction with glycosyl halides: see, *e.g.*, ref 2c, p 166; ref 2f, p 46; and ref 8c. Schneider and Bhacca[5a] report the presence of traces of cholesterol-α-D-glucosiduronate AcMe in a preparation of the β anomer from bromo sugar **1** and Ag_2O in benzene at room temperature.

(19) Various absorption bands, mainly in the range of *ca.* 800–950 cm^{-1}, have been attributed to the α- and β-glycosidic linkages. Recently, J. J. Schneider, *Carbohyd. Res.*, **12**, 369 (1970), has reported a band at 1146–1140 cm^{-1} as diagnostic for the α anomers of a series of anomeric, steroidal, aliphatic glucuronide triacetate methyl esters, and glucoside tetraacetates. Effects of the environment of the glycosidic bond on the band contours between 1125 and 1000 cm^{-1} has been demonstrated by E. Smakula, J. H. Leftin, and H. H. Wotiz, *J. Amer. Chem. Soc.*, **81**, 1708 (1959). It was only in this region that obvious and consistent differences existed between the steroidal, anomeric glycosides isolated by the present authors.

(20) Poor absolute agreement was obtained between the calculated and found molecular rotations. However, the figures clearly differentiate between the anomers when considered as differences in orders of magnitude: W. Klyne in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955, p 98.

(21) L. D. Hall, *Advan. Carbohyd. Chem.*, **19**, 51 (1964).

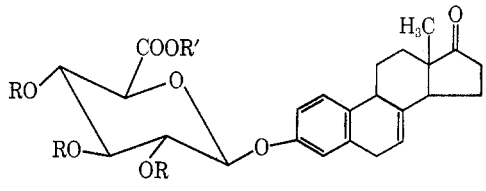
(22) Various Lewis acids have been used to anomerize β- to α-glycosides: E. Pacsu, J. Janson, and B. Lindberg, "Methods in Carbohydrate Chemistry," Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., 1963, p 376. Schneider[19] has recently applied the $TiCl_4$ reagent to the preparation of a series of acetylated, steroidal α-glucuronides, and glucosides from the acetylated β-glucuronide. Several metal halides, including cadmium chloride, have also been shown to cause glycoside anomerization and O → *N*-glycosyl rearrangement: D. Thacker and T. L. V. Ulbricht, *Chem. Commun.*, 122 (1967).

(23) This is believed to be the first reported example of a *C*-glycosyl derivative of a steroid. For a review of *C*-glycosyl derivatives, see L. J. Haynes, *Advan. Carbohydrate Chem.*, **20**, 357 (1965).

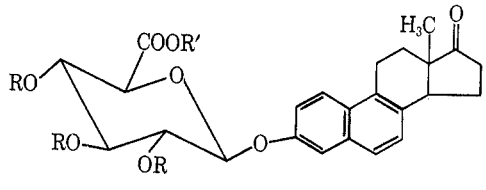
(24) A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, New York, N. Y., 1964, p 95.

(25) A. Prox, *Tetrahedron*, **24**, 3697 (1968), discusses the mass spectrum of *C*-glucoside derivatives of flavonoids.

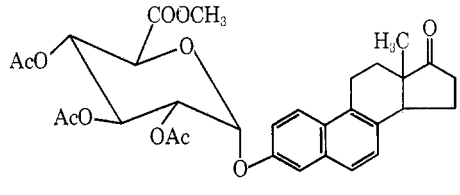
results indicated an exceptionally stable sugar–steroid linkage. The nmr spectrum of **19** revealed two sugar acetates in normal positions at δ 2.01 and 2.09, and a third methyl group far upfield at δ 1.3. This value was appreciably outside the range of δ 1.67–1.75 reported for the C-2 acetate methyl signal of *C*-glucosyl derivatives of flavonoids.[26,27] Thus, some doubt remained about the actual structure of the unknown. Methylation of the product with diazomethane gave **20** whose nmr spectrum proved easier to interpret than that of the phenol **19**. Furthermore the sugar C-2 acetate methyl was found at δ 1.62 which was more consistent with the previously mentioned values[26,27] for related compounds. Two pairs of ortho aromatic protons were clearly evident in the nmr spectrum of **20** and this showed that the sugar must be substituted at C-4 of the steroid.[28] By using field-sweep decoupling techniques, it was shown that doublets at δ 8.0 and 7.25 were associated, and these were assigned to the C-1 and C-2 protons, respectively. Similarly, doublets at δ 8.38 and 7.42 were associated and these were assigned to the C-6 and C-7 protons, respectively. Significantly, the doublet at δ 8.38 was very diffuse at ambient temperature (40°), whereas at 90° it sharpened to a normal pattern. This indicated steric hindrance between the sugar and C-6 proton and confirmed the assignment for the aromatic protons. The configuration of the glucuronosyl–steroid bond is the only structural feature which remains in doubt because of the obscurity of the C-1 sugar proton[29] in the nmr spectrum. The formation of **19** in significant yield indicates that cadmium carbonate may have value for the preparation[30] of other *C*-glycosyl derivatives of reactive aromatic compounds.



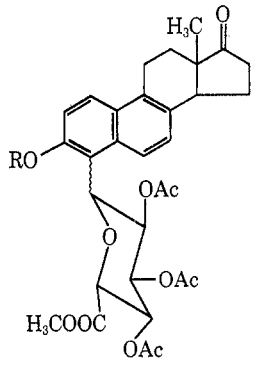
14, R = Ac; R′ = CH_3
15, R = H; R′ = Na



16, R = Ac; R′ = CH_3
17, R = H; R′ = Na



18



19, R = H
20, R = CH_3

Additional information on the nature and limitations of the cadmium carbonate promoted glycosidation reaction was obtained during the course of our investigations. While the glucuronidation of estrone was run successfully in benzene, toluene, or chlorobenzene, no reaction was obtained in toluene when dimethylacetamide (17%), sulfolane (17%), or pyridine (1 equiv with respect to the halo sugar) were present. The reason for failure of the reaction under these conditions is unknown, but complexing[31] of the cadmium halide with the polar additive is one possibility. Evidence suggesting that the cadmium halide, or a cadmium halide species, produced in the reaction is the actual catalyst[12] was obtained in experiments with the chloro sugar **2**.[32] Thus, when **2** was used in the glucuronidation of estrone, the yield (75%) of product compared favorably to that obtained with bromo sugar **1**. However, initiation of the reaction, as manifested by a change in color[33] (colorless to pale tan and finally red), did not occur until 30 min after the addition of chloro sugar was started. In reactions with bromo sugar, color change was evident after 3–5 min. It seemed likely that the longer induction period was the result of the greater thermal stability of the chloro sugar and, hence, the longer time required for the formation of trace amounts of hydrogen halide and, hence, of cadmium halide before the reaction could become autocatalytic. Indeed, when the reaction mixture was treated with a trace of anhydrous hydrogen chloride prior to the dropwise addition of chloro sugar **2**, the formation of product was evident after 5 min. Moreover, when the cadmium carbonate was pretreated with excess anhydrous hydrogen chloride, the glucuroni-

(26) W. E. Hillis and D. H. S. Horn, *Aust. J. Chem.*, **18**, 531 (1965).

(27) At a recent conference, however, it was learned that the C-2 acetate methyl of the 1-glucosyl-Ac_4 derivative of naphthalene[30a] resonates at δ 1.5: L. J. Haynes, CIC–ACS Joint Conference, Toronto, May 1970.

(28) This is also the expected position of substitution by analogy with electrophilic substitution in naphthalene which goes almost exclusively in the α position: H. Zollinger, "Azo and Diazo Chemistry," Interscience, New York, N. Y., 1961, p 231, and references therein.

(29) The C-1,2,3,4 sugar protons of **20** are grouped together over the range of δ 5.42–5.92.

(30) Aromatic *C*-glycosyl derivatives have been prepared *via* glycosyl halides using (a) Friedel–Crafts catalysts or Grignard reagents [W. A. Bonner, *Advan. Carbohyd. Chem.*, **6**, 251 (1951)] and (b) metal alkoxides. See, for example, V. K. Bhatia and T. R. Seshadri, *Tetrahedron Lett.*, 1741 (1968).

(31) Stable complexes of cadmium halides with a variety of nucleophiles have been reported. For example, see, J. C. Barnes and C. S. Duncan, *J. Chem. Soc. A*, 1746 (1969), and B. Paul and D. V. R. Rao, *Can. J. Chem.*, **46**, 334 (1968).

(32) W. D. S. Bowering and T. E. Timell, *J. Amer. Chem. Soc.*, **82**, 2827 (1960).

(33) That the appearance of color did in fact correspond to the initiation of the reaction was verified by tlc monitoring of the reaction.

dation reaction again proceeded smoothly, and to completion, with little or no apparent induction period. In view of this, it was surprising to find that commercial anhydrous $CdCl_2$ or $CdBr_2$ were ineffective as catalysts.[34] The reason for this is obscure and requires further investigation. Inasmuch as the reaction is apparently heterogeneous, one possibility could be a difference in surface properties, such as surface area. Thus, when different brands[35] of cadmium carbonate were used, it was observed that the particle size and, hence, surface area of the catalyst had a large effect on the rate of the reaction. This effect is consistent with a heterogeneous reaction.

The free glucuronides were obtained by alkaline hydrolysis of the acetylated products, avoiding vigorous conditions. Thus, the glucuronide triacetate methyl esters were treated with a 1.0 molar excess of aqueous sodium hydroxide in methanol or ethanol at room temperature for 1 hr. Generally the product precipitated and was readily crystallized from aqueous ethanol. However, attempts to crystallize equilin-3-glucuronide (**15**) were unsuccessful. The product was purified reasonably well (tlc evidence) by precipitation but failed to give a satisfactory elemental analysis. Estrone-3-β-D-glucoside tetraacetate (**7**) was treated with saturated ammonia–methanol solution overnight at 4° to give the deblocked glucoside **8** in good yield.

Experimental Section[36]

Procedure A. Trial Glucuronidations of Estrone.—A mixture of 250 mg (0.925 mmol) of estrone and 1.5 mmol of the catalyst in 14 ml of toluene was distilled until *ca.* 2 ml of toluene had been removed. The mixture was cooled slightly and 600 mg (1.51 mmol) of bromo sugar[10] **1** was added. The mixture was stirred and refluxed for 1 hr and then filtered through Celite and evaporated to an oil. Half of the crude product was purified by preparative tlc (system A) and material from the product band was crystallized once from CH_2Cl_2–EtOH. With NaOAc, 3.0 mmol were used. With CdS as catalyst, the mole ratio of estrone: bromo sugar:CdS was 1:2:4. In the reaction with HgO–$HgBr_2$ (1.5:0.07 mmol) the product was partially obscured on the tlc plate by strongly uv-absorbing materials, and it was necessary to purify the product again by tlc.

Procedure B. General Glucuronidation Procedure Using $CdCO_3$.—All equipment and reagents were thoroughly dried before use. A mixture of the steroid (5.0 mmol), cadmium carbonate[35] (1.72 g, 10.0 mmol), and 100 ml of toluene was distilled until *ca.* 25 ml of toluene had been removed, thus ensuring dryness of the reagents and equipment. A solution of the bromo sugar[10] **1** (3.97 g, 10.0 mmol) in 100 ml of toluene was added dropwise to the stirred mixture over 1 hr and an equal volume of toluene was distilled from the flask at the same rate. Distillation was continued for a further 0.5 hr during which an equal volume (50 ml) of makeup toluene was added dropwise. The mixture was filtered through a pad of Celite, and the filtrate was evaporated to an oil. The oil was dissolved in dimethylformamide or acetone (25–50 ml) and poured into water (200 ml). The mixture was filtered through a pad of Celite and the precipitate was washed on the filter with water and then dissolved in CH_2Cl_2. The resulting solution was dried and evaporated to give the crude product as an easily crystallizable oil. Additional purification is outlined below under the individual compounds.

Methyl [17-Oxoestra-1,3,5(10)-trien-3-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (4).—The crude product (3.15 g) obtained from the general procedure B using 1.35 g (5.0 mmol) of estrone was crystallized three times from CH_2Cl_2–EtOH to give 2.09 g (71%) colorless plates, mp 222–230°. Analytical material was obtained by further purification of a sample by tlc (system A). The product was crystallized twice from CH_2Cl_2–EtOH to give colorless plates: mp 230–233°; $[\alpha]^{25}D$ +55° (*c* 0.70, $CHCl_3$); ir (KBr) 1754 (ester + C-17, C=O), 1493 (aromatic), 1220 (ester COC), 1094 sh (glycosidic COC), 1040 cm^{-1} (ester); uv max (MeOH) 217, 278 mμ (ε 10,500, 1470); nmr ($CDCl_3$) δ 7.18 (d, 1, H-1), 6.78 (m, 2, H-2,4), 5.25 (m, 4, H-1′,2′,3′,4′), 4.17 (m, 1, H-5′), 3.73 (s, 3, COOMe), 2.85 (m, 2′, C-6 CH_2), 2.05 (s, 9, three OAc), 0.90 (s, 3, H-18); mass spectrum[17] *m/e* 127, 155, 197, 257, 317, 215, 270, *m/e* 365, 407, 393, 527, 467, 586 (M^+), 425, 423, 555, 569.

Anal. Calcd for $C_{31}H_{38}O_{11}$: C, 63.47; H, 6.53. Found: C, 63.24; H, 6.44.

17-Oxoestra-1,3,5(10)-trien-3-yl-2′,3′,4′,6′-tetra-*O*-acetyl-D-glucopyranoside, β and α Anomers (7 and 9).—The crude product (3.43 g) obtained from the general procedure B using 1.35 g (5.0 mmol) of estrone and 3.95 g (9.61 mmol) of acetobromoglucose[37] was crystallized once from CH_2Cl_2–EtOH to give 1.85 g (61%) colorless needles, mp 212–216°. Analytical material was obtained as follows. The product, in CH_2Cl_2 solution, was filtered through a bed of Magnesol (20 g) using 300 ml of CH_2Cl_2 wash. Material from the filtrate was crystallized once from CH_2Cl_2–EtOH to give the β anomer **7** as fine colorless needles: 1.55 g; mp 214–217°; $[\alpha]^{25}D$ +65° (*c* 1.02, $CHCl_3$); ir (KBr) 1761 (acetate and C-17, C=O), 1504 (aromatic), 1232 (acetate COC), 1081 sh, 1067 sh (glycosidic COC), 1047 cm^{-1} (acetate); uv max (MeOH) 215, 275 mμ (ε 11,400, 1560); nmr ($CDCl_3$) δ 7.18 (d, 1, H-1), 6.78 (m, 2, H-2,4), 5.17 (m, 4, H-1′,2′,3′,4′), 4.23 (m, 2, H-6′CH_2), 3.90 (m, 1, H-5′), 2.85 (m, 2, H-6 CH_2), 2.08, 2.05, 2.03 (t, 12, four OAc), 0.90 (s, 3, H-18); mass spectrum[17] *m/e* 169, 109, 331, 127, 170, 145, 139, 271, *m/e* 379, 365, 353, 407, 421, 541, 600 (M^+), 527, 437.

Anal. Calcd for $C_{32}H_{40}O_{11}$: C, 64.00; H, 6.71. Found: C, 63.73; H, 6.62.

All mother liquors from the isolation of **7** were evaporated to a glass which was purified by partition chromatography on Celite using heptane:chloroform:methanol:water 50:1:10:2.5. In order of elution, there was obtained estrone 3-acetate (37 mg), α anomer **9** (244 mg), and β anomer **7** (404 mg) as uncrystallized glasses. The α-anomer fraction was crystallized from ether–hexane to give 194 mg (6.5%) colorless crystals, mp 95–100° (partial) and 125–135° (final). Material of analytical purity was obtained by an additional crystallization from ether–hexane followed by a final crystallization from isopropyl ether. Slow cooling gave colorless needles solvated with isopropyl ether. Drying overnight at 100° *in vacuo* gave unsolvated needles of α anomer **9**: mp 133–136°; $[\alpha]^{25}D$ +203° (*c* 0.64, $CHCl_3$); ir (KBr) 1757 (acetate + C-17, C=O), 1499 (aromatic), 1229 (acetate COC), 1075 sh (glycosidic COC), 1044 cm^{-1} (acetate); uv max (MeOH) 215, 275 mμ (ε 11,000, 1380); nmr ($CDCl_3$ +

(34) Interestingly, in a similar case Helferich[8a] observed that, whereas $HgBr_2$ was practically inactive as a glycosidation catalyst, it had a net rate-enhancing effect when used in conjunction with the active catalyst $Hg(CN)_2$ (in methanol).

(35) When $CdCO_3$ from Fisher Scientific Co. was used for the glucuronidation of estrone, the reaction was incomplete at 48% yield of **4**, whereas under the same conditions Baker Analyzed $CdCO_3$ gave a 71% yield of product. However, when the Fisher material was vigorously ground in a mortar, it gave a 58% yield of product. In view of this, the reaction time and/or amount of $CdCO_3$ outlined in the general procedure (see Experimental Section) may have to be increased for optimum yields in some cases.

(36) The $CdCO_3$ used throughtout was Baker Analyzed material unless otherwise indicated.[35] The toluene was distilled over CaH_2 and stored over molecular sieves (Linde, type 4A). Magnesol (Food Machinery Chemical Corp.) is a hydrous magnesium sulicate, decolorizing adsorbent. Celite (Johns-Manville Co.) is a diatomaceous silica filter aid. Solutions were dried over anhydrous Na_2SO_4 and all evaporations were under reduced pressure. In crystallizations from CH_2Cl_2–EtOH, the material was dissolved in CH_2Cl_2 and absolute EtOH added to the boiling solution until all of the CH_2Cl_2 had been removed. Thin layer chromatography (tlc) was carried out on 250-μ-thick, silica gel GF Uniplates (Analtech Inc.). In preparative tlc, 20 × 20 cm plates with 500–1000-μ layers of silica gel GF were used. For the acetylated glucuronides, the plates were developed twice with 5% acetone–benzene (system A) unless otherwise indicated. A useful system for the deblocked glucuronides was $CHCl_3$–HOAc–H_2O, 30:35:3 (system B). Visualization was by uv light and 10% phosphomolybdic acid–methanol spray. Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. The infrared spectra were run in pressed KBr disks on a Perkin-Elmer Model 21 spectrophotometer. Ultraviolet spectra were run on a Cary Model 11 recording spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Nuclear magnetic resonance spectra were determined on a Varian A-60 spectrometer with tetramethylsilane as internal standard. The mass spectra were determined at 70 eV on an Associated Electrical Industries MS-9 instrument.

(37) C. E. Redemann and C. Niemann, "Organic Syntheses," Collect. Vol. III, Wiley, New York, N. Y., 1955, p 11.

C_6D_6) δ 7.00 (m, 3, H-1,2,4), 5.82 (t, 1, J = 9.0 Hz, H-3′), 5.75 (d, 1, J = 3.5 Hz, H-1′), 5.38 (m, 1, H-4′), 5.08 (q, 1, J = 10.0 Hz, H-2′), 4.12 (m, 2, H-5′ CH_2), 2.73 (m, 2, H-6 CH_2), 1.88, 1.87, 1.85, 1.82 (q, 12, four OAc), 0.88 (s, 3, H-18); mass spectrum[17] m/e 169, 109, 331, 127, 170, 270, 145 139, 271, m/e 600 (M^+), 379, 365, 353, 541, 421, 395, 407, 437, 439.

Anal. Calcd for $C_{32}H_{40}O_{11}$: C, 64.00; H, 6.71. Found: C, 63.82; H, 6.60.

Methyl [17β-Formyloxyestra-1,3,5(10)-trien-3-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (10).—The crude product (3.4 g) obtained from the general procedure B using 1.50 g (5.0 mmol) of estradiol-17β-formate[38] was crystallized three times from CH_2Cl_2–EtOH and then filtered through a bed of Magnesol (24 g) using 300 ml of CH_2Cl_2 wash. The filtrate was evaporated, and the residue was crystallized twice from CH_2Cl_2–EtOH to give **10** as colorless crystals (2.18 g, 71%) of analytcal purity and mp 260–263°: $[\alpha]^{25}D$ 0° (c 0.73, $CHCl_3$); ir (KBr) 1764 (ester C=O), 1724 (formate C=O), 1502 (aromatic), 1222 (ester COC), 1183 sh (formate COC), 1096 (glycosidic COC), 1045 cm^{-1} (ester); uv max (MeOH) 215, 278 mμ (ε 13,000, 1540); nmr ($CDCl_3$) δ 8.10 (s, 1, OCHO), 7.17 (d, 1, H-1), 6.78 (m, 2, H-2,4), 5.25 (m, 4, H-1′,2′,3′,4′), 4.77 (m, 1, H-17), 4.23 (m, 1, H-5′), 3.73 (s, 3, COOMe), 2.82 (m, 2, C-6 CH_2), 2.03 (s, 9, three OAc), 0.85 (s, 3, H-18); mass spectrum[17] m/e 127, 155, 197, 257, 317, 215, 300, m/e 395, 437, 423, 557, 497, 585, 571, 616 (M^+).

Anal. Calcd for $C_{32}H_{40}O_{12}$: C, 62.32; H, 6.54. Found: C, 62.31; H, 6.55.

Methyl [16α,17β-Diformyloxyestra-1,3,5(10)-trien-3-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (12).—The crude product (1.8 g) obtained from half the scale of the general procedure B using 861 mg (2.5 mmol) of estriol-16α,17β-diformate[38] was crystallized three times from CH_2Cl_2–EtOH in presence of activated carbon to provide 1.08 g (65%) of colorless crystals, mp 225–230°. The analytical sample was obtained by an additional crystallization from CH_2Cl_2–EtOH followed by filtering the product through a small bed of Magnesol, in CH_2Cl_2 solution. The resulting material was given a final crystallization from CH_2Cl_2–EtOH to afford **12** as colorless needles: mp 225–233°; $[\alpha]^{25}D$ −34° (c 0.71, $CHCl_3$); ir (KBr) 1770 (ester C=O), 1736 (formate C=O), 1506 (aromatic), 1232 (ester COC), 1172 (formate COC), 1100 sh, 1075 sh (glycosidic COC), 1047 cm^{-1} (ester); uv max (MeOH) 215, 275 mμ (ε 14,200, 1650); nmr ($CDCl_3$) δ 8.10 (s, 1, C-17 OCHO), 8.01 (s, 1, C-16 OCHO), 7.17 (d, 1, H-1), 6.77 (m, 2, H-2,4), 5.25 (m, 6, H-1′,2′,3′,4′ + H-16,17), 4.18 (m, 1, H-5′), 3.72 (s, 3, COOMe), 2.82 (m, 2, H-6 CH_2), 2.03 (s, 9, three OAc), 0.88 (s, 3, H-18); mass spectrum[17] m/e 155, 127, 317, 257, 197, 215, 344, m/e 439, 386, 481, 601, 467, 541, 660 (M^+), 497, 629, 569, 615.

Anal. Calcd for $C_{33}H_{40}O_{14}$: C, 59.99; H, 6.10. Found: C, 59.82; H, 6.08.

Methyl [17-Oxoestra-1,3,5(10),7-tetraen-3-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (14).—The crude product (3.38 g) obtained from the general procedure B using 1.34 g (5.0 mmol) of equilin was crystallized three times from CH_2Cl_2–EtOH to give 2.0 g (68.5%, two crops) of colorless needles, mp 154–159°. The analytical sample was obtained as follows. The product was crystallized from CH_2Cl_2–EtOH and then filtered through a bed of Magnesol in methylene chloride solution. Material from the filtrate was crystallized twice from ether–hexane to give **14** as colorless crystals: mp 165–169°; $[\alpha]^{25}D$ +119° (c 0.80, $CHCl_3$); ir (KBr) 1764 (ester + C-17 C=O), 1504 (aromatic), 1224 (ester COC), 1099 (glycosidic COC), 1046 cm^{-1} (ester); uv max (MeOH) 275, 283 mμ (ε 1520, 1400); nmr ($CDCl_3$) δ 7.20 (d, 1, H-1), 6.88 (m, 2, H-2,4), 5.55 (m, 1, H-7), 5.30 (m, 4, H-1′,2′,3′,4′), 4.22 (m, 1, H-5′), 3.75 (s, 3, COOMe), 3.47 (m, 2, H-6 CH_2), 2.05, 2.03 (d, 9, three OAc), 0.78 (s, 3, H-18); mass spectrum[17] m/e 187, 155, 317, 257, 197, 215, 268, 266, m/e 363, 582, 584 (M^+), 405, 525, 391, 465.

Anal. Calcd for $C_{31}H_{36}O_{11}$: C, 63.69; H, 6.20. Found: C, 63.59; H, 6.10.

Methyl [17-Oxoestra-1,3,5(10),6,8-pentaen-3-yl-2′,3′,4′-tri-*O*-acetyl-D-glucopyranosid]uronate, β and α Anomers (16 and 18). Methyl [3-Hydroxy-17-oxoestra-1,3,5(10),6,8-pentaen-4-yl-2′,3′,4′-tri-*O*-acetyl-1′-deoxy-1′-ξ-D-glucopyran]uronate (19).—The crude product (3.05 g) obtained from the general procedure B using 1.33 g (5.0 mmol) of equilenin was crystallized from CH_2Cl_2–EtOH (30–35 ml) to give 1.38 g, mp 210–216°, of almost pure β-glucuronide **16** by tlc (system A). On concentration of the mother liquor to 10–15 ml, there was obtained 267 mg of crystalline material, mp 248–262° dec, which was substantially pure *C*-glucuronosyl derivative **19** by tlc (system A).

The β-glucuronide **16** was crystallized again from CH_2Cl_2–EtOH to provide 1.35 g (46%) of colorless crystals, mp 212–216°. Analytical material was obtained by filtering the product through a bed of Magnesol using CH_2Cl_2 as eluent followed by a final crystallization from CH_2Cl_2–EtOH to give **16** as colorless crystals: mp 215–218°; $[\alpha]^{25}D$ +13° (c 0.87, $CHCl_3$); ir (KBr) 1767 (ester + C-17 C=O), 1631, 1608 (aromatic), 1229 (ester COC), 1099 (glycosidic COC), 1044 cm^{-1} (ester); uv max (MeOH), 232 (ε 74,500), 269 (4600), 280 (5530), 291 (4360), 318 (1750), 332 mμ (2040); nmr ($CDCl_3$) δ 7.93 (d, 1, J = 8.0 Hz, H-6), 7.27 (m, 3, H-2,4,7), 5.37 (m, 4, H-1′,2′,3′,4′), 4.27 (m, 1, H-5′), 3.75 (s, 3, COOMe), 2.07 (s, 9, three OAc), 0.78 (s, 3, H-18); mass spectrum[17] m/e 155, 127, 317, 197, 257, 266, 215, 223, 210, m/e 582 (M^+), 361, 522, 523, 403, 462, 463.

Anal. Calcd for $C_{31}H_{34}O_{11}$: C, 63.90; H, 5.88. Found: C, 63.76; H, 5.76.

The *C*-glucuronosyl compound **19** was crystallized twice from CH_2Cl_2–EtOH to give 205 mg (7.0%) of analytical material as off-white crystals: mp 265–268°; $[\alpha]^{25}D$ +83° (c 0.86 $CHCl_3$); ir (KBr) 3436 (OH), 1767 (ester + C-17 C=O), 1626, 1608 (aromatic), 1224 (ester COC), 1105, 1037 cm^{-1} (ester); uv max (MeOH) 236 (ε 61,750), 276 (4950), 287 (6700), 299 (6110), 333 (3500), 345 mμ (3780); uv max (0.1 *N* NaOH, MeOH), 216 (ε 51,250), 247 (50,400), 279 (7000), 290 (7560), 301 sh (4360), 363 mμ (4360); nmr ($CDCl_3$) δ 8.10 (br m, 1, H-6), 7.92 (d, 1, J = 9.5 Hz, H-1), 7.28 (d, 1, J = 8.5 Hz H-7), 7.18 (d, 1, J = 9.5 Hz, H-2), 5.57 (m, 4, H-1′,2′,3′,4′), 4.33 (m, 1, H-5′), 3.80 (s, 3, COOMe), 2.08, 2.00 (d, 6, C-3′, C-4′ OAc), 0.13 (s, 3, C-2′ OAc) 0.72 (s, 3, H-18); mass spectrum[17] m/e 582 (M^+), 319, 343, 361, 303, 403, 331, 279, 308, 294, 238, 251, 197, m/e 522, 540, 420, 480.

Anal. Calcd for $C_{31}H_{34}O_{11}$: C, 63.90; H, 5.88. Found: C, 63.64; H, 5.76.

Filtrates from the crystallization of **16** and **19** were evaporated and the residue (*ca.* 1.6 g) was chromatographed on silica gel (150 g, Mallinckrodt SilicAR CC-7, 100–200 mesh). Elution with 5% then 10% acetone–hexane gave 40 mg (2.6%) of material which on crystallization from ether–hexane afforded tan crystals, mp 140–153°, of equilenin 3-acetate by ir. Elution with 15% acetone–hexane gave the next fraction of 328 mg which on crystallization from acetone–benzene provided 192 mg (14.5%), mp 240–250°, of yellow solid which was equilenin by ir and tlc. Elution with 20% acetone–hexane gave 125 mg of crude α anomer **18** (see preparation of analytical material below). Increasing the polarity of the eluent to 30% acetone–hexane provided 203 mg of material as the next component. This was crystallized from CH_2Cl_2–EtOH to give 84 mg (2.9%), mp 206–212°, of additional β-glucuronide **16**. Continuing with 30% acetone–hexane gave 363 mg of solid which on crystallization from CH_2Cl_2–EtOH provided 225 mg (7.7%), mp 254–265°, of tan crystals of additional *C*-glucuronosyl derivative **19**.

The α anomer **18**, obtained above, was purified further by tlc (developed six times with 25% acetone–hexane) to give 66 mg (2.3%) of product. Crystallization from ether followed by two crystallizations from EtOH provided the α-glucuronide **18** as colorless needles: mp 226–230°; $[\alpha]^{25}D$ +183° (c 0.45 $CHCl_3$); ir (KBr) 1757 (ester + C-17 C=O), 1629, 1608 (aromatic), 1229 (ester COC), 1078 sh (glycosidic COC), 1053 cm^{-1} (ester); uv max (MeOH) 232 (ε 71,600), 268 (4360), 280 (5240), 291 (4075), 317 (1750), 332 mμ (1800); nmr ($CDCl_3$) δ 7.93 (d, 1, J = 9.5 Hz, H-1), 7.65 (d, 1, J = 8.5 Hz, H-6), 7.37 (m, 3, H-2, 4, 7), 5.98 (d, 1, J = 3.5 Hz, H-1′), 5.82 (t, 1, J = 10.0 Hz, H-3′), 5.30 (t, 1, J = 10.0 Hz, H-4′), 5.15 (q, 1, J = 10.0 Hz, H-2′), 4.50 (d, 1, J = 10.0 Hz, H-5′), 3.72 (s, 3, COOMe), 2.07, 2.05, 2.03 (t, 9, three OAc), 0.79 (s, 3, H-18); mass spectrum[17] m/e 155, 127, 266, 197, 257, 317, 215, 156, 210, 223, 209, 582 (M^+), m/e 522, 523, 403.

Anal. Calcd for $C_{31}H_{34}O_{11}$: C, 63.90; H, 5.88. Found: C, 63.75; H, 5.77.

Methyl [3-Methoxy-17-oxoestra-1,3,5(10),6,8-pentaen-4-yl-2′,3′,4′-tri-*O*-acetyl-1′-deoxy-1′-ξ-D-glucopyran]uronate (20).—To a solution of 150 mg (0.257 mmol) of the *C*-glucuronosyl phenol **19** in 2 ml of CH_2Cl_2 and 2 ml of MeOH was added a solution of diazomethane (*ca.* 2.7 mmol) in 10 ml of ether. The

(38) J. P. Dusza and J. P. Joseph (unpublished work) prepared these compounds by selective formylation of the parent steroid with 88% formic acid on the steam bath.

solution was stored overnight in the dark at room temperature. Evaporation of the solution gave a solid which was only 50% reacted by tlc. The product was reacted again as above using 10 ml of 1:1 CH_2Cl_2:CH_3OH and a solution of diazomethane (*ca.* 4.1 mmol) in 15 ml of ether. The excess CH_2N_2 was decomposed with a small amount of HOAc and the solution evaporated. The residue was purified by tlc (developed twice with 10% acetone–benzene) and crystallized from CH_2Cl_2–EtOH and finally from CH_2Cl_2–CH_3OH to give 104 mg of **20** as a colorless solid: mp 290–303°; $[\alpha]^{25}D$ −3.5° (*c* 0.86 $CHCl_3$); ir (KBr) 1754 (ester + C-17 C=O), 1621, 1600 (aromatic), 1241, 1218 (ester COC), 1103 sh, 1034 cm^{-1} (ester); uv max (MeOH) 236 (ε 94,000), 276 (6260), 287 (8640), 299 (7750), 330 (4470), 344 mμ (4780); nmr ($CDCl_3$) δ 8.38 (br m, 1, H-6), 8.00 (d, 1, *J* = 9.5 Hz, H-1), 7.42 (d, 1, *J* = 8.5 Hz, H-7), 7.25 (d, 1, *J* = 9.5 Hz, H-2), 5.67 (m, 4, H-1′,2′,3′,4′), 4.28 (m, 1, H-5′), 3.96 (s, 3, C-3 OMe), 3.74 (s, 3, COOMe), 2.08, 2.01 (d, 6, C-3′,4′ OAc), 1.62 (s, 3, C-2′ OAc), 0.78 (s, 3, H-18); nmr ($CDCl_3$ + C_6D_6 at 90°) δ 8.37 (d, 1, *J* = 9.0 Hz, H-6); mass spectrum[17] *m/e* 375, 596 (M^+), 143, 357, 309, 127, 417, *m/e* 477, 536, 527, 610, 553, 565.

Anal. Calcd for $C_{32}H_{36}O_{11}$: C, 64.42; H, 6.08. Found: C, 64.40; H, 6.06.

Hydrolysis of Acetylated Glycosides. Sodium [17-Oxoestra-1,3,5(10)-trien-3-yl-β-D-glucopyranosid]uronate (5).—To a suspension of 1.17 g (2.0 mmol) of the glucuronide triacetate methyl ester **4** in 30 ml of absolute MeOH was added 2.0 ml (10.0 mmol) of 5 *N* NaOH. The mixture was stirred at room temperature for 1 hr then coevaporated several times with EtOH to a small volume. The product was filtered and crystallized from 90% aqueous EtOH to give 635 mg (64%) colorless plates, 270–288° dec. The analytical sample was obtained by an additional crystallization from 90% EtOH to give **5** as colorless plates: 287–297° dec; $[\alpha]^{25}D$ +26° (*c* 0.92, H_2O); ir (KBr) 3413 (OH), 1739 (C-17 C=O), 1618 (carboxylate C=O), 1497 (aromatic), 1404 (COO^-), 1060 cm^{-1} (hydroxyl CO); uv max (MeOH) 215, 275 mμ (ε 9700, 1240); nmr (DMSO-d_6) δ 7.20 (d, 1, H-1), 6.83 (m, 2, H-2,4), 5.40 (br s, 3, OH), 4.80 (m, 2, sugar H's), 0.83 (s, 3, H-18).

Anal. Calcd for $C_{24}H_{29}O_8Na \cdot 1^1/_2H_2O$: C, 58.17; H, 6.51; H_2O, 5.45. Found: C, 58.46; H, 6.25; H_2O,[39] 4.1.

Sodium [17β-Hydroxyestra-1,3,5(10)-trien-3-yl-β-D-glucopyranosid]uronate (11).—To a suspension of 1.23 g (2.0 mmol) of the formylglucuronide triacetate methyl ester, **10**, in 60 ml of absolute MeOH was added 2.8 ml (14.0 mmol) of 5 *N* NaOH. The mixture was stirred at room temperature for 1 hr then coevaporated several times with EtOH to small volume and filtered. The product was crystallized from 70% aqueous acetone to give 666 mg (65%) of **11** as colorless crystals, 270–279° dec. An additional crystallization from aqueous acetone gave analytical material as colorless plates: 271–280° dec; $[\alpha]^{25}D$ −13° (*c* 0.99 H_2O); ir (KBr) 3390 (OH), 1616 (carboxylate C=O), 1497 (aromatic), 1418 (COO^-), 1060 cm^{-1} (hydroxyl CO); uv max (MeOH) 215, 275 mμ (ε 10,000, 1300); nmr (DMSO-d_6) δ 7.17 (d, 1, H-1), 6.78 (m, 2, H-2,4), 6.55 (m, 1, C-17 OH?), 5,35 (s, 3, OH), 4.77 (m, 1, sugar H?), 4.53 (m, 1, sugar H?), 0.67 (s, 3, H-18).

Anal. Calcd for $C_{24}H_{31}O_8Na \cdot 2^1/_2H_2O$: C, 55.91; H, 7.04; H_2O, 8.5. Found: C, 56.15; H, 6.81; H_2O, 7.2.

Sodium [16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-yl-β-D-glucopyranosid]uronate (13).—A mixture of 660 mg (1.0 mmol) of the diformylglucuronide triacetate methyl ester, **12**, 30 ml of absolute EtOH and 1.4 ml (7.0 mmol) of 5 *N* NaOH was stirred at room temperature for 1 hr. The mixture was filtered and the product was crystallized from 70% aqueous EtOH to give 339 mg (65%) of colorless needles, 265–275° dec. Antlytical material was obtained by an additional crystallization from aqueous EtOH to give **13** as colorless needles: 270–280° dec; $[\alpha]^{25}D$ −23° (*c* 0.75, H_2O); ir (KBr) 3390 (OH) 1613 (carboxylate C=O), 1497 (aromatic), 1416 (COO^-), 1053 cm^{-1} (hydroxyl CO); uv max (MeOH) 215, 276 mμ (ε 10,000, 1300); nmr (DMSO-d_6) δ 7.17 (d, 1, H-1), 6.80 (m, 2, H-2,4), 5.35 (s, 3, OH), 4.82 (m, 3, sugar H?), 0.68 (s, 3, H-18).

Anal. Calcd for $C_{24}H_{31}O_9Na \cdot 2H_2O$: C, 55.17; H, 6.76; H_2O, 6.9. Found: C, 54.86; H, 6.64; H_2O, 3.90.

Sodium [17-Oxoestra-1,3,5(10),7-tetraen-3-yl-β-D-glucopyranosid]uronate (15).—To a solution of 468 mg (0.8 mmol) of the glucuronide triacetate methyl ester, **14**, in 24 ml of absolute EtOH and 3 ml of CH_2Cl_2 was added 0.8 ml (4.0 mmol) of 5 *N* NaOH. The mixture was stirred at room temperature for 1 hr and filtered to give 270 mg (72%) of tan solid. All attempts to crystallize the product from a variety of systems resulted in the formation of a gum. The product was dissolved in refluxing anhydrous MeOH, treated with activated carbon, and filtered. The filtrate was concentrated to 5 ml and diluted at reflux with 5 ml of acetone. The resulting precipitate, plus a second crop from the filtrate, was precipitated again from methanol to give 139 mg of **15** as a tan solid which had only a trace of organic impurity by tlc (system B). Melting point of the product was 214–228°; $[\alpha]^{25}D$ +108° (*c* 0.97, H_2O); ir (KBr) 3367 (OH), 1739 (C-17 C=O), 1618 (carboxylate C=O), 1506 (aromatic), 1414 (COO^-), 1063 cm^{-1} (hydroxyl CO); uv max (MeOH) 276, 283 (sh) mμ (ε 1740, 1550); nmr (DMSO-d_6) δ 7.22 (d, 1, H-1), 6.88 (m, 2, H-2,4), 5.52 (br s, 2, H-7 + sugar H?), 4.82 (m, 1, H-1′), 3.33 (m, 10, sugar OH + H_2O?), 0.68 (s, 3, H-18).

Anal. Calcd for $C_{24}H_{27}O_8Na \cdot H_2O$: C, 59.50; H, 6.03; Na, 4.74; H_2O, 3.72. Found: C, 58.09; H, 5.86; Na, 5.92; H_2O, 2.7.

Sodium [17-Oxoestra-1,3,5(10),6,8-pentaen-3-yl-β-D-glucopyranosid]uronate (17).—A mixture of 582 mg (1.0 mmol) of the glucuronide triacetate methyl ester, **16**, 30 ml of absolute EtOH and 1.0 ml of 5 *N* NaOH (5.0 mmol) was stirred at room temperature for 1 hr. The mixture was evaporated to small volume and filtered, and the product was crystallized from 40% aqueous EtOH to give 300 mg (65%) of colorless crystals, mp 275–290°. The product was crystallized from 60% aqueous EtOH followed by two crystallizations from water (approx 2 ml) to provide an analytical sample (129 mg) of **17** as colorless needles: mp 288–295° dec; $[\alpha]^{25}D$ −8.5° (*c* 0.99, H_2O); ir (KBr) 3367 (OH), 1739 (C-17 C=O), 1623, 1600 (carboxylate C=O), 1508 (aromatic), 1429 (COO^-), 1064 cm^{-1} (hydroxyl CO); uv max (MeOH) 232 (ε 79,000), 268 (4825), 280 (5550), 290 (4100), 318 (1690), 333 mμ (1810); nmr (DMSO-d_6) δ 7.93 (d, 1, *J* = 9.5 Hz H-1), 7.67 (d, 1, *J* = 8.5 Hz H-6), 7.35 (m, 3, H-2, 4, 7), 5.17 (m, 3, sugar OH), 0.67 (s, 3, H-18).

Anal. Calcd for $C_{24}H_{23}O_8Na \cdot H_2O$: C, 59.74; H, 5.64; Na, 4.77; H_2O, 3.73. Found: C, 59.90; H, 5.57; Na, 4.46; H_2O, 4.4.

17-Oxoestra-1,3,5(10)-trien-3-yl-β-D-glucopyranoside (8).—To a solution of 348 mg (0.58 mmol) of the glucoside tetraacetate, **7**, in 1.0 ml of CH_2Cl_2 was added 20 ml of methanol which had been saturated with anhydrous ammonia at 0°. The solution was refrigerated at 4° overnight and then evaporated to a colorless glass. The glass was triturated with water and filtered, and the resulting solid was crystallized from 25% aqueous EtOH to give 238 mg (94%) of **8** in analytical purity as colorless crystals: mp 150–170°; $[\alpha]^{25}D$ +63° (*c* 0.72, MeOH); ir (KBr) 3390 (OH), 1724 (C-17 C=O), 1499 (aromatic), 1072 cm^{-1} (hydroxyl C–O); uv max (MeOH) 275, 283 (sh) mμ (ε 1470, 1310); nmr (DMSO-d_6) δ 7.22 (d, 1, H-1), 6.80 (m, 2, H-2,4), 5.20–4.33 (m, 5, sugar H′), 0.86 (s, 3, H-18); mass spectrum[17] *m/e* 270, 146, 185, 162, 172, *m/e* 312, 323, 317, 365, 432 (M^+).

Anal. Calcd for $C_{24}H_{32}O_7 \cdot {}^1/_4H_2O$: C, 65.96; H, 7.49. Found: C, 65.96; H, 7.65.

Glucuronidation of Estrone in Presence of Cadmium Halides. A. $CdCl_2$ Generated *in Situ*.—A small amount (*ca.* 10 bubbles) of anhydrous HCl was passed into a stirred and distilling mixture of 270 mg (1.0 mmol) of estrone, 345 mg (2.0 mmol) of $CdCO_3$, and 20 ml of toluene. A solution of 705 mg (2.0 mmol) of chloro sugar **2**[32] in 20 ml of toluene was then added dropwise over 1 hr according to general procedure B except on $^1/_5$th the scale. The reaction was monitored by tlc (system A), samples being taken every 5 min for the first 30 min and then every 10 min for the next 90 min. A trace of product was evident after 5 min and definitely present after 10 min. At this time a change in color (colorless to pale tan) of the mixture was observed. The product uniformly increased with time, and the estrone decreased until the reaction was complete after a total of 90 min.

In another reaction, using the same quantities of reagents, anhydrous HCl was bubbled through the distilling mixture of estrone, $CdCO_3$, and toluene for 1 hr during which makeup toluene (20 ml) was added. A solution of the chloro sugar in toluene was added dropwise over 1 hr as in the general procedure B. The results, as determined by tlc monitoring, were indistinguishable from those above except that the initial color change occurred during treatment with HCl. In an identical

(39) The Karl Fischer water analyses are approximate values due to the nature of the determination; however, they are given here as additional justification for the inclusion of water in the molecular formula.

reaction which was not pretreated with anhydrous HCl, the appearance of color and of product (tlc monitoring) was not evident until 25–30 min. Repetition of this showed a color change after 22 min. After 90 min a sample (1/4) of the mixture was purified by tlc (system A), and the product was crystallized from CH_2Cl_2–EtOH to give 110 mg (75%) of **4** as colorless plates, mp 227–230°.

B. CdX_2 Added.—Estrone (270 mg, 1.0 mmol) was glucuronidated with chloro sugar **2** (705 mg, 2.0 mmol) using $CdCO_3$ (345 mg, 2.0 mmol) and 18 mg (0.1 mmol) of anhydrous $CdCl_2$ (Coleman and Bell Co., Norwood, Ohio) according to the general procedure B. The mixture changed color after 30 min. Separation of a sample (1/4) of the mixture by tlc (system A) and crystallization of the product from CH_2Cl_2–EtOH gave 91.7 mg (62.5%) of **4**, mp 226–231°.

In another experiment, estrone (250 mg, 0.925 mmol) was reacted with bromo sugar **1** according to procedure A using 410 mg (1.51 mmol) of anhydrous $CdBr_2$ (Alfa Inorganics) and 208 mg (1.50 mmol) of anhydrous K_2CO_3 as the catalyst–acid acceptor system. However, no product was evident by tlc (system A) and work-up of the mixture gave back 79% of the estrone and 96% of the bromo sugar.

Registry No.—**4**, 27537-72-0; **5**, 15087-01-1; **7**, 27610-08-8; **8**, 25591-03-1; **9**, 27610-09-9; **10**, 27537-75-3; **11**, 14982-12-8; **12**, 27537-76-4; **13**, 15087-06-6; **14**, 27570-87-2; **15**, 27610-12-4; **16**, 27537-77-5; **17**, 27537-78-6; **18**, 27537-79-7; **19**, 27537-80-0; **20**, 27537-81-1; cadmium carbonate, 513-78-0.

Acknowledgments.—We thank Louis M. Brancone and associates for the elemental analyses and William Fulmor and associates for the spectral data. Special thanks go to Dr. George E. Van Lear for the mass spectra and to George O. Morton for his most helpful interpretations of the nmr data.

Synthesis of Tobacco Mosaic Virus Protein Sequence 81–85[1]

GEORGE R. PETTIT* AND WILLIAM R. JONES

Department of Chemistry, Arizona State University, Tempe, Arizona 85281

Received July 10, 1970

The 81–85 segment of tobacco mosaic virus protein has been prepared by two different synthetic approaches. Synthesis of the protected pentapeptide *N*-benzyloxycarbonyl-L-threonyl-L-alanyl-L-leucyl-L-leucyl-glycine hydrazide corresponding to TMV protein 81–85 was accomplished employing as key step coupling of *N*-Z-Thr-Ala azide with Leu-Leu-Gly-OMe. The product was identical with the same pentapeptide obtained by a Merrifield solid-phase synthesis.

Synthesis of the tobacco mosaic virus protein would represent an important step toward the first total synthesis of an organism capable of replication. With this objective in view, we began a program concerned with synthesis of, at that time (1962) known, segments of the TMV protein. By 1964 the complete structure of TMV protein had been proposed with reasonable certainty.[2] Subsequently, the 120–124[3a] (solution polymer method) and 151–154[3b] (fragment condensation) units were prepared in our laboratory and units 42–46[4a] and 103–112[4b] have been prepared (solid phase technique) elsewhere. Concurrent with preparation of TMV protein fragments, we have been using certain of these peptides in an immunological[5] study of steroidal peptides[6a] and in preparation of alkaloidal peptides.[6b] The preparation reported herein of the fully protected pentapeptide *N*-Z-Thr-Ala-Leu-Leu-Gly hydrazide corresponding to TMV protein sequence 81–85 was accomplished by both conventional methods of peptide synthesis in solution and by a Merrifield solid-phase[7] synthesis.

Synthesis of pentapeptide **6** by a fragment condensation approach proceeded as follows. Condensation of *tert*-butoxycarbonyl-L-leucine with glycine methyl ester proceeded well in the presence of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI)[8] and gave protected dipeptide **1**. Attempts at cleaving the *tert*-butoxycarbonyl group of dipeptide **1** using trifluoroacetic acid and hydrogen chloride in methylene chloride or in methanol gave a two-component mixture. However, use of 98% formic acid[9] gave a pure product (**2**). A mixed carbonic anhydride[10] coupling procedure was used to condense *tert*-butoxycarbonyl-L-leucine with dipeptide ester **2**. By this means, the protected tripeptide **3a** was obtained in good yield. By contrast, the use of dicyclohexylcarbodiimide in methylene chloride afforded a low yield of tripeptide **3a**. The dipeptide fragment *N*-Z-Thr-Ala-OMe (**4**) was conveniently obtained as described by Hofmann, *et al.*,[11] using dicyclohexylcarbodiimide. Noteworthy at this stage of the synthesis was the observation that *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (WRK)[12] in acetonitrile or nitromethane, or EDCI in methylene chloride, led to consistently low yields of the protected dipeptide **4**. Hydrazinolysis of Z-Thr-Ala-OMe **4** to

(1) Contribution XI of the series Structural Biochemistry. For part X, see P. Brown and G. R. Pettit, *Org. Mass Spectrom.*, **3**, 67 (1970). We are grateful to the National Science Foundation for financial support by Grants GB-4939 and GB-8250.

(2) G. Funatsu, A. Tsugita, and H. Fraenkel-Conrat, *Arch. Biochim. Biophys.*, **105**, 25 (1964).

(3) (a) B. Green and L. Garson, *J. Chem. Soc. C*, 401 (1969); (b) G. R. Pettit and S. K. Gupta, *J. Chem. Soc.*, 1208 (1968).

(4) (a) J. D. Young, C. Y. Leung, and W. A. Rombauts, *Biochemistry*, **7**, 2475 (1968). (b) J. M. Stewart, J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *ibid.*, **5**, 3396 (1966); J. D. Young, E. Benjamini, J. M. Stewart, and C. Y. Leung, *ibid.*, **6**, 1455 (1967).

(5) For example, the 93–112 and 102–112 units of TMV protein have been reported to be antigenic areas: J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *ibid.*, **5**, 1481 (1966); E. Benjamini, M. Shimizu, J. D. Young, and C. Y. Leung, *ibid.*, **8**, 2242 (1969).

(6) (a) G. R. Pettit, R. L. Smith, and H. Klinger, *J. Med. Chem.*, **10**, 145 (1967); G. R. Pettit and N. H. Rogers, *J. Org. Chem.*, manuscript in preparation. (b) G. R. Pettit and S. K. Gupta, *J. Chem. Soc. C*, 1208 (1968).

(7) R. B. Merrifield, *Advan. Enzymol. Relat. Subj. Biochem.*, **32**, 221 (1969).

(8) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961). The abbreviations for amino acids, peptides, and coupling reagents are those recommended by the IUPAC Commission on nomenclature, *Biochemistry*, **5**, 2485 (1966), and those adopted by G. R. Pettit in "Synthetic Peptides," Vol. I, Van Nostrand–Reinhold, New York, N. Y., 1970.

(9) B. Halpern and D. E. Nitecki, *Tetrahedron Lett.*, 3031 (1967).

(10) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **89**, 5012 (1967).

(11) K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *ibid.*, **87**, 611 (1965).

(12) R. B. Woodward and D. J. Woodman, *J. Org. Chem.*, **34**, 2742 (1969); R. B. Woodward, R. A. Olofson, and H. Mayer, *Tetrahedron*, **S8**, 321 (1966).