[CONTRIBUTION FROM THE ORGANIC CHEMISTRY DEPARTMENT, RESEARCH DIVISION, ABBOTT LABORATORIES]

Lactones Derived from 17β-Hydroxyestran-16β-ylacetic Acids

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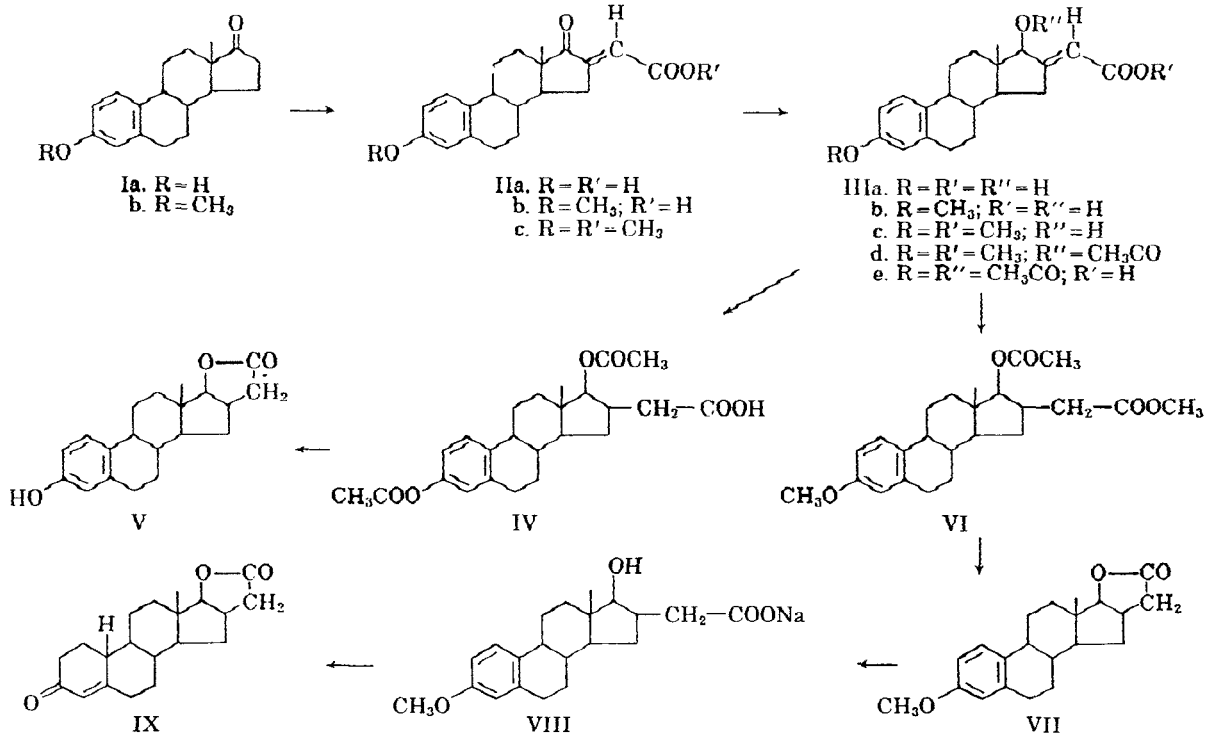
The condensation of estrone with glyoxylic acid gave rise to 3-hydroxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid. The latter served as an intermediate to obtain 3,17β-dihydroxy-1,3,5(10)-estratrien-16β-ylacetic acid lactone and its 3-methoxy analog. Appropriate reduction and hydrolysis led to the desired 17β-hydroxy-3-oxo-4-estren-16β-ylacetic acid lactone.

In an effort to alter the hormonal properties of estrone and related substances, we have prepared several lactones having a two-carbon side chain at position 16 of the estrane nucleus. Our initial success with the glyoxylic acid condensation in the preparation of lactones in the androstane series[1] prompted us to extend this work to the estrane derivatives.

The available 3-hydroxy-17-oxo-1,3,5(10)-estratriene (estrone, Ia) and its methyl ether (Ib) were condensed with glyoxylic acid to give 3-hydroxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid (IIa) and 3-methoxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid (IIb). The method of Newman, Sagar, and Cochrane[2] for this type of glyoxylic acid condensation was used. The reaction of IIa with dimethyl sulfate in an alkaline solution led to the isolation of IIb and methyl 3-methoxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetate (IIc). The methyl ester IIc was also prepared by esterification of IIb.

Proof that condensation had occurred at C-16 of 3-hydroxy-17-oxo-1,3,5(10)-estratriene (Ia) was clearly established by the ultraviolet absorption pattern of the methylated derivative IIb and by the infrared absorption spectra of IIa, IIb, and IIc. The possibility of condensation at C-2 of Ia had been suggested by the work of Patton,[3] but in our case the principal reaction proved to be at C-16, analogous to that which had occurred in the androstane series.[1]



Reduction of IIa with sodium borohydride afforded 3,17β - dihydroxy - 1,3,5(10) - estratrien-16-ylidenacetic acid (IIIa), and the latter (IIIa) was converted into the methyl ether IIIb, which was also obtained after the reduction of IIb with sodium borohydride. Esterification of IIIb yielded methyl 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16-ylidenacetate (IIIc), which upon acetylation

(1) P. Kurath and W. Cole, *J. Org. Chem.*, 26, 1939 (1961).

(2) M. S. Newman, W. C. Sagar, and C. C. Cochrane, *J. Org. Chem.*, 23, 1832 (1958).

(3) T. L. Patton, *Chem. & Ind. (London)*, 923 (1959); *J. Org. Chem.*, 25, 2148 (1960).

gave rise to methyl 17β-acetoxy-3-methoxy-1,3,5-(10)-estratrien-16-ylidenacetate (IIId). When IIIa was acetylated, 3,17β-diacetoxy-1,3,5(10)-estratrien-16-ylidenacetic acid (IIIe) was obtained. Selective hydrogenation of IIId or IIIe led to the isolation of methyl 17β-acetoxy-3-methoxy-1,3,5(10)-estratrien-16β-ylacetate (VI) and 3,17β-diacetoxy-1,3,5(10)-estratrien-16β-ylacetic acid (IV) respectively.

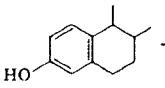
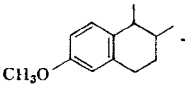
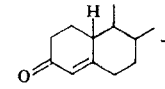
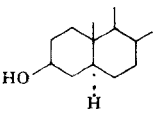
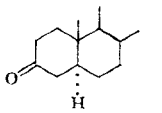
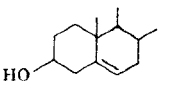
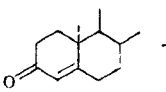
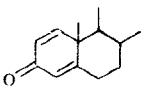
The assignment of β-configuration to the substituents on C-17 in structures IIIa–IIIe, and on C-16 and C-17 in structures IV and VI is based on the same reasons as in the previously described androstane series. The fact that IIIa was isolated in the form of the free acid rather than the corresponding lactone allows the tentative assignment of the *trans* configuration[1] to the side chain of IIIa. This assignment of *trans* configuration also applies to the related structures II and III.

After the treatment of IV or VI with potassium hydroxide and subsequently hydrochloric acid, the 3,17β - dihydroxy - 1,3,5(10) - estratrien - 16β-ylacetic acid lactone (V) and the 17β-hydroxy-3 - methoxy - 1,3,5(10) - estratrien - 16β - ylacetic acid lactone (VII), respectively, were obtained. The lactone VII was converted almost quantitatively to sodium 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16β-ylacetate (VIII) and the latter (VIII) was subject to a Birch reduction[4] under conditions previously employed by Cella, Brown, and Burtner[5] in a similar case. The crude intermediate from the Birch reduction was treated with acid to yield the desired 17β-hydroxy-3-oxo-4-estren-16β-ylacetic acid lactone (IX).

The stereochemical relationship of the lactones described in this paper with those of the androstane series previously described was further verified by comparison of optical rotations. In each series there is a small positive shift (ΔM_D +60° ± 30°) in molecular rotation caused by the 17β-hydroxy-16β-ylacetic acid lactone structure, as compared with the parent 17β-hydroxy steroid having no substituent at C-16. The molecular rotation differences are presented in the accompanying table.

The lactones and intermediates are being assayed for possible endocrine activities. At this time it appears that several of them have antiandrogenic properties (*e.g.*, IIa, IIIa, IIIe, V and IX).

COMPARISON OF MOLECULAR ROTATIONS

Partial Structure			ΔM_D
	+215°[a]	+303°[c]	+88° (di)[e]
	+220°[a]	+280°[c]	+60° (chf)[f]
	+151°[b]	+238°[c]	+87° (chf)
	+12°[a]	+73°[d]	+61° (ethanol, chf)
	+87°[a]	+159°[d]	+72° (chf)
	−160°[a]	−125°[d]	+35° (chf)
	+340°[a]	+371°[d]	+31° (chf)
	+63°[a]	+95°[d]	+32° (chf)

[a] J.-P. Mathieu and A. Petit, *Pouvoir Rotatoire Naturel, I. Stéroïdes*, Masson et Cie., Paris 1956. [b] A. L. Wilds and N. A. Nelson, *J. Am. Chem. Soc.*, 75, 5366 (1953). [c] See Experimental part. [d] See ref. 1. [e] Dioxane. [f] Chloroform.

EXPERIMENTAL[6-10]

3-Hydroxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid (IIa). To the cooled solution of glyoxylic acid, generated from 21.56 g. of methyl 2,2-dimethoxyacetate in 40 ml. of water,[11] a solution of 21.62 g. of estrone (Ia) and 13.55 g. of sodium hydroxide pellets in 220 ml. of water and 200 ml. of methanol was added.[1,3] This reaction mixture was agitated at room temperature for 18 hr. and then warmed to a gentle reflux for 2 hr. The cooled suspension was acidified with 600 ml. of 2*N* hydrochloric acid and diluted with 300 ml. of water. The slurry was extracted with three 300-ml. portions of methylene chloride. After the methylene chloride solution was washed with water, dried, and evaporated, the residue was recrystallized from acetone to yield 0.525 g. of estrone (Ia). The crude acid IIa, which remained in the water suspension, was collected on a filter, washed with several small amounts of water, and recrystallized from

(4) A. J. Birch, *Quart. Revs.*, 4, 69 (1950).

(5) J. A. Cella, E. A. Brown, and R. R. Burtner, *J. Org. Chem.*, 24, 743 (1959).

(6) The melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus unless stated otherwise. The optical rotations were measured in a 1-dm. tube in chloroform or dioxane solutions. The values have a limit of error of ±2°.

(7) We wish to thank Mr. Elmer Shelberg and his staff for microanalyses.

(8) The infrared spectra were recorded on a Perkin-Elmer infrared spectrophotometer Model 21 using chloroform and carbon tetrachloride solutions or potassium bromide pellets. We are indebted to Mr. William H. Washburn and his associates for the recording and interpretation of the infrared spectra.

(9) We wish to thank Mr. Frank Chadde for the measurement of the ultraviolet spectra.

(10) In the catalytic reductions we had the assistance of Messrs. Morris Freifelder and George R. Stone, to both of whom we wish to express our thanks.

(11) W. J. Close, L. R. Swett, and C. W. Nordeen, *J. Org. Chem.*, 26, 3423 (1961).

methanol-water. A first crop of 18.65 g. (72%) of IIa, m.p. 302–304° dec. (capillary), was isolated. A second crop amounted to 2.866 g., m.p. 292–294°.

A sample was recrystallized from methanol-water for analysis, m.p. 307–308° dec. (capillary); $[\alpha]_D^{24}$ +80° (*c*, 0.79 dioxane); λ_{max}^{KBr} 2.93 μ, 3.13 μ, 3.6–4.0 μ, 5.83–5.90 μ, 6.05 μ, 6.15 μ, 6.29 μ, 6.66 μ.

Anal. Calcd. for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.64; H, 6.91.

3-Methoxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid (IIb). A) *From 3-hydroxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid* (IIa). A solution of 0.625 g. of the acid IIa and 0.486 g. of sodium hydroxide pellets in 5.5 ml. of water and 5.5 ml. of methanol was treated with 1.1 ml. of dimethyl sulfate in 5.5 ml. of methanol according to the procedure of Schindler.[12] The reaction mixture was transferred to a separatory funnel, diluted with 200 ml. of 0.05*N* sodium hydroxide solution and extracted with methylene chloride. The methylene chloride extract was washed with 0.1*N* sodium hydroxide solution and water, dried, and evaporated to leave 0.237 g. of neutral material (for work-up see A under IIc).

The alkaline solution was acidified and extracted with methylene chloride. The organic phase was washed with water, dried over anhydrous magnesium sulfate, filtered, and evaporated to leave 0.467 g. of acid. The residue was recrystallized from methanol-water to yield 0.366 g. of IIb, m.p. 245–247° dec.

A sample was further recrystallized from methanol-water to a constant melting point of 250–251° dec.; $[\alpha]_D^{25}$ + 82° (*c*, 0.60 dioxane); λ_{max}^{KBr} 3.6–4.0 μ, 5.77 μ, 5.89 μ, 6.05 μ, 6.19 μ, 6.69 μ.

Anal. Calcd. for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 74.17; H, 7.41.

B) *From 3-methoxy-17-oxo-1,3,5(10)-estratriene* (Ib). To a cold solution of glyoxylic acid, prepared from 2.22 g. of *d*-tartaric acid and 3.16 g. of trisodium periodate (para) in 20 ml. of water and 0.3 ml. of concd. sulfuric acid, were added 4.20 g. of Ib, 2.22 g. of sodium hydroxide pellets, 40 ml. of water, and 37 ml. of methanol. The reaction mixture was treated as previously described,[1,2] diluted with 250 ml. of water, and extracted with methylene chloride. The organic phase was washed with 0.2*N* sodium hydroxide solution and water, dried and evaporated to leave, after recrystallization from acetone, 3.123 g. of recovered Ib.

The above alkaline washes were acidified with 5*N* sulfuric acid and extracted with methylene chloride. The organic solution was washed with water, dried, filtered, and evaporated to leave a residue of 0.920 g. of acid. The compound was recrystallized from methanol to give 0.566 g. of IIb, m.p. 250–251° dec.; $[\alpha]_D^{25}$ +82° (*c*, 0.82 dioxane); λ_{max}^{KBr} 3.6–4.0 μ; 5.77 μ, 5.89 μ, 6.05 μ, 6.19 μ, 6.69 μ.

Anal. Calcd. for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 73.82; H, 6.97.

The products from preparations A and B are identical. The low yield resulting from procedure B is due to the low solubility of the starting material Ib in the reaction medium.

The ultraviolet spectrum of the condensation product IIb was recorded: $\lambda_{max}^{CH_3OH}$ 230 mμ (ε 17,200), 278 mμ (ε 3,100), 286 mμ (ε 2,500); $\lambda_{min}^{CH_3OH}$ 273 mμ (ε 2,900), 284 mμ (ε 2,400); shoulder at 240 mμ (ε 14,000). When the ultraviolet spectrum of the above sample IIb was measured using an equimolecular solution of Ib as the reference solution, only the expected absorption for the 17-keto-16-ylidenacetic acid chromophore[1] was observed: $\lambda_{max}^{CH_3OH}$ 240 mμ (ε 13,200).

Methyl 3-methoxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetate (IIc). A) *From 3-hydroxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid* (IIa). The neutral material (0.237 g.) from the above described methylation of IIa was obtained in plates, m.p. 130–131°, from a fairly concentrated methanol solution. When the compound was allowed to crystallize from a more dilute methanol solution, fine needles, m.p. 149–150°, were obtained. The dimorphic crystals of IIc gave superimposable infrared spectra in chloroform solution and furnished correct analyses. The above high melting sample had $[\alpha]_D^{24}$ +78° (*c*, 0.91 chloroform); $\lambda_{max}^{CHCl_3}$ 5.80 μ, 6.01 μ, 6.19 μ, 6.66 μ.

Anal. Calcd. for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.22; H, 7.16.

B) *From 3-methoxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid* (IIb). A solution of 0.38 g. of the keto acid IIb in 4 ml. of methanol and 0.1 ml. of concd. hydrochloric acid was esterified in the presence of 2,2-dimethoxypropane as previously described.[1,13] The reaction mixture was worked up as usual and the crude residue was recrystallized from a dilute methanol solution to yield 0.233 g. of fine needles of IIc, m.p. 149–150°; $[\alpha]_D^{25}$ +78° (*c*, 1.27 chloroform); $\lambda_{max}^{CHCl_3}$ 5.80 μ, 6.01 μ, 6.19 μ, 6.66 μ.

Anal. Calcd. for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.28; H, 7.64.

The products of the above preparations A and B were found to be identical.

3,17β-Dihydroxy-1,3,5(10)-estratrien-16-ylidenacetic acid (IIIa). The solution of 15.60 g. of 3-hydroxy-17-oxo-1,3,5-(10)-estratrien-16-ylidenacetic acid (IIa) in 1400 ml. of methanol was reduced with 7.2 g. of sodium borohydride following the experimental procedure previously employed in a similar case.[1] The compound was recrystallized from acetone. A first crop of 6.146 g. of IIIa, m.p. 320–321° dec. (capillary), was obtained. Upon concentration of the mother liquors a second crop of 8.103 g., m.p. 317–320° dec. (capillary) was isolated. The yield was 91%.

Purification for analysis gave a sample with a decomposition point of 321–323° (capillary); $[\alpha]_D^{25}$ −1° (*c*, 0.84 dioxane); λ_{max}^{KBr} 2.84 μ, 3.15 μ, 3.6–4.0 μ, 5.89 μ, 6.01 μ, 6.15 μ, 6.29 μ, 6.67 μ.

Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.14; H, 7.37. Found: C, 72.88; H, 7.35.

17β-Hydroxy-3-methoxy-1,3,5(10)-estratrien-16-ylidenacetic acid (IIIb). A) *From 3,17β-dihydroxy-1,3,5(10)-estratrien-16-ylidenacetic acid* (IIIa). To the solution of 14.249 g. of IIIa and 12.15 g. of sodium hydroxide pellets in 125 ml. of methanol and 125 ml. of water a solution of 25 ml. of dimethyl sulfate in 45 ml. of methanol was added dropwise.[12] The reaction temperature was kept at 45–50° during the addition. After the dropping funnel was rinsed once with 75 ml. of methanol, the reaction mixture was stirred at 50–55° for 3 hr., at room temperature for 2 hr. and then allowed to stand overnight. Sodium hydroxide pellets (2.13 g.) were added and the mixture was refluxed for 2 hr. The resulting solution was diluted to 2000 ml. with water and acidified with 300 ml. of 5*N* sulfuric acid. The acidic suspension was warmed on the steam bath for 15 min., allowed to cool, and filtered. The solid was washed with several small amounts of water and recrystallized from methanol-water to yield 12.85 g. (86%) of IIIb, m.p. 230–231°.

A part of the above compound was recrystallized to a constant melting point of 235–236°; $[\alpha]_D^{25}$ 0° (*c*, 1.00 dioxane); λ_{max}^{KBr} 3.10 μ, 3.7–3.8 μ, 5.90 μ, 6.09 μ, 6.20 μ, 6.67 μ.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.72; H, 7.77.

B) *From 3-methoxy-17-oxo-1,3,5(10)-estratrien-16-ylidenacetic acid* (IIb). The sodium borohydride reduction of IIb was carried out as described above for IIIa. The reaction product was recrystallized first from methanol-water and then from acetone–petroleum ether (b.p. 90–95°). An analytical sample melted at 236–237°; $[\alpha]_D^{25}$ 0° (*c*, 0.50 dioxane); λ_{max}^{KBr} 3.10 μ, 3.7–3.8 μ, 5.90 μ, 6.09 μ, 6.20 μ, 6.67 μ.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.70; H, 8.03.

The compound prepared by procedure A was identical to the product of procedure B.

(12) O. Schindler, *Helv. Chim. Acta*, **43**, 754 (1960).

(13) N. B. Lorette and J. H. Brown, Jr., *J. Org. Chem.*, **24**, 261 (1959).

Methyl 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16-ylidenacetate (IIIc). A solution of 11.75 g. of IIIb in methanolic hydrochloric acid was esterified in the presence of 2,2-dimethoxypropane under previously reported conditions.[1,13] The product was recrystallized from methanol to yield 10.182 g. (83%) of IIIc, m.p. 173–174°. A further 0.934 g. of less pure compound, m.p. 161–164°, was obtained from the mother liquors.

Further recrystallization of a part of the first crop gave an analytical sample, m.p. 177–178°; $[\alpha]_D^{24}$ −4° (*c*, 1.36 chloroform); $\lambda_{max}^{CHCl_3}$ 2.8 μ, 2.90 μ, 5.84 μ, 6.0 μ, 6.21 μ, 6.67 μ.

Anal. Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.13; H, 8.21.

Methyl 17β-acetoxy-3-methoxy-1,3,5(10)-estratrien-16-ylidenacetate (IIId). A solution of 9.76 g. of the above prepared hydroxy ester IIIc in 70 ml. of pyridine and 35 ml. of acetic anhydride was allowed to stand at room temperature overnight and worked up as usual. Recrystallization from methanol gave a first crop of 9.855 g. (90%) of IIId, m.p. 139–140°. Upon concentration of the mother liquors a second crop of 0.571 g., m.p. 129–130°, was isolated.

A sample was recrystallized again for analysis, m.p. 139–140°; $[\alpha]_D^{25}$ −9° (*c*, 1.10 chloroform); $\lambda_{max}^{CCl_4}$ 5.74 μ, 5.81 μ, 6.00 μ, 6.22 μ, 6.70 μ.

Anal. Calcd. for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59. Found: C, 72.13; H, 7.60.

3,17β-Diacetoxy-1,3,5(10)-estratrien-16-ylidenacetic acid (IIIe). A solution of 5.60 g. of IIIa in 40 ml. of pyridine and 20 ml. of acetic anhydride was allowed to stand at room temperature overnight. The mixture was cooled in an ice bath, diluted carefully with 20 ml. of water, and allowed to stand for 10 min. The solution was then diluted with 250 ml. of ice cold 2*N* hydrochloric acid; the precipitate was collected on a filter and washed with several small portions of water. The product was recrystallized from methanol-water to give a first crop of 4.846 g. of IIIe, m.p. 199–201°. A second crop amounted to 1.380 g., m.p. 195–196°, bringing the yield to 88%.

A sample was recrystallized from acetone–petroleum ether (b.p. 90–95°) for analysis, m.p. 199–200°; $[\alpha]_D^{25}$ +7° (*c*, 1.34 chloroform); $\lambda_{max}^{CCl_4}$ 3.6–4.0 μ, 5.64 μ, 5.71 μ, 5.91 μ, 6.04 μ, 6.70 μ.

Anal. Calcd. for $C_{24}H_{28}O_6$: C, 69.89; H, 6.84. Found: C, 70.05; H, 6.95.

3,17β-Diacetoxy-1,3,5(10)-estratrien-16β-ylacetic acid (IV). The solution of 2.062 g. of IIIe in 100 ml. of methanol and 5 ml. of water was reduced over a 2% ratio of platinum oxide. The catalyst was separated by filtration and the resulting clear solution was concentrated to give, after cooling, 1.885 g. (91%) of IV, m.p. 217–219°.

An analytical sample melted at 218–219°; $[\alpha]_D^{24}$ +40° (*c*, 1.16 chloroform); $\lambda_{max}^{CHCl_3}$ 3.6–4.0 μ, 5.65–5.75 μ, 5.83 μ, 6.23 μ, 6.71 μ.

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.57; H, 7.09.

3,17β-Dihydroxy-1,3,5(10)-estratrien-16β-ylacetic acid lactone (V). A solution of 1.63 g. of IV was treated with 2.00 g. of potassium hydroxide pellets in 75 ml. of methanol and 7.5 ml. of water. The reaction mixture was worked up as previously described in a similar case[1] and the crude product was recrystallized from acetone to give 1.046 g. (85%) of V, m.p. 306–308° dec. (capillary).

A sample was recrystallized to a constant melting point of 310–311° dec. (capillary); $[\alpha]_D^{24}$ +97° (*c*, 0.63 dioxane); λ_{max}^{KBr} 3.02 μ, 5.71 μ, 6.15 μ, 6.27 μ, 6.66 μ.

Anal. Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.96; H, 7.79.

Methyl 17β-acetoxy-3-methoxy-1,3,5(10)-estratrien-16β-ylacetate (VI). The solution of 3.99 g. of IIId in 190 ml. of methanol and 10 ml. of water was reduced over 0.1 g. of platinum oxide. The catalyst was separated by filtration and the solvent was evaporated. The crystalline residue of 3.94 g. was recrystallized from methanol to yield 3.232 g. (81%) of VI, m.p. 103–104°. A second crop of 0.278 g. of crystalline material, m.p. 96–97°, was isolated from the mother liquors.

The analytical sample had a melting point of 104–105°; $[\alpha]_D^{25}$ +41° (*c*, 0.94 chloroform); $\lambda_{max}^{CCl_4}$ 5.75 μ, 6.20 μ, 6.67 μ.

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.90; H, 7.80.

17β-Hydroxy-3-methoxy-1,3,5(10)-estratrien-16β-ylacetic acid lactone (VII). The solution of 6.18 g. of VI was hydrolyzed and lactonized. The reaction product was recrystallized from acetone and gave 4.024 g. (80%) of VII, m.p. 216–218°. A second crop of 0.422 g., m.p. 207–210°, was obtained upon concentration of the mother liquors.

An analytical sample had a m.p. of 220–221°; $[\alpha]_D^{24}$ +86° (*c*, 1.30 chloroform); $\lambda_{max}^{CHCl_3}$ 5.66 μ, 6.20 μ, 6.66 μ, 8.53 μ.

Anal. Calcd. for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found: C, 76.99; H, 8.12.

Sodium 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16β-ylacetate (VIII). The mixture of 4.024 g. of the lactone VII and 31 ml. of a 2*N* aqueous sodium hydroxide solution in 240 ml. of methanol was refluxed gently on the steam bath for 30 min. The reaction mixture was diluted with 250 ml. of water and concentrated under reduced pressure to approximately 200 ml. The resulting suspension was cooled, the crystalline compound was collected on a filter and washed with a total of 240 ml. of ice cold water. The dried sodium salt (VIII) amounted to 4.326 g. (96%), m.p. 242–246° dec. The filtrate was made acidic by adding 50 ml. of 2*N* hydrochloric acid. The precipitate was collected and recrystallized from acetone to yield 0.10 g. of the starting material VII, m.p. 214–216°.

17β-Hydroxy-3-oxo-4-estren-16β-ylacetic acid lactone (IX). A suspension of 1.62 g. of VIII in 26 ml. of tetrahydrofuran and 26 ml. of *t*-butyl alcohol was diluted to about 170 ml. with liquid ammonia. The mixture was cooled in a Dry Ice–acetone bath and a total of 0.93 g. of lithium wire was added to the stirred solution in small pieces over a period of 15 min. in accordance with the Birch reduction procedure as described by Cella, Brown, and Burtner.[5] The reaction mixture was stirred and cooled for 5 hr. With continued cooling and stirring 5 ml. of anhydrous ethyl alcohol was added dropwise to the blue solution. Twenty-five minutes later a second portion of 5 ml. of anhydrous ethyl alcohol was added and cooling and stirring were continued until the dark color of the solution disappeared after 20 min. The cooling bath was removed and the ammonia was allowed to evaporate in a stream of nitrogen.

The reaction mixture was diluted with 300 ml. of distilled water, brought to *p*H 5 by the addition of 200 ml. of 1*N* oxalic acid and extracted with 700 ml. of ether. The water phase was separated and extracted with two successive portions of 400 ml. of ether. The ether extracts were then washed with water, combined, and dried over anhydrous magnesium sulfate, filtered, and concentrated. Petroleum ether (b.p. 62–67°) was gradually added and the solution was concentrated until crystals formed. The cool mixture was filtered to give 1.245 g. of crystalline product, m.p. 151–153° (capillary); λ_{max}^{KBr} 2.97 μ, 3.7–4.0 μ, 5.85 μ, 5.98 μ, 6.19 μ, 6.66 μ. Concentration of the mother liquors led to a second crop of 0.106 g., m.p. 108–112°; an oily residue of 0.186 g. was obtained after evaporation of the mother liquors.

The above infrared data show that the reduction was incomplete. Several repeat experiments using different reaction times did not correct this.

A mixture of 0.963 g. of the above Birch reduction product (m.p. 151–153°), 9.25 ml. of 2*N* hydrochloric acid, 4.6 ml. of water, and 46 ml. of methanol was refluxed under nitrogen for 40 min. After the addition of 13.9 ml. of 2*N* sodium hydroxide solution, the reaction mixture was warmed for an additional 15 min. under nitrogen. This was followed by the addition of 92 ml. of water and the solution was concentrated to approximately 40 ml. under vacuum. The resulting slurry was diluted with 40 ml. of water and 40 ml. of 10%

hydrochloric acid was added. The mixture was warmed on the steam bath for 15 min., allowed to cool, and extracted with ether. The extract was washed with water, dried over anhydrous magnesium sulfate, filtered, and evaporated to leave a solid residue of 0.916 g., which was purified by chromatography on 100 g. of silica gel. From the benzene-ether (1:1) eluates a total of 0.186 g. of crude lactone VII was obtained. The compound was recrystallized from acetone to give 0.098 g., m.p. 214–216°; admixture of this compound with a reference sample of VII did not depress the melting point.

The eluates with ether and ether-acetone (95:5) gave, after the evaporation of the solvent, 0.747 g. of impure 17β-hydroxy-3-oxo-4-estren-16β-ylacetic acid lactone (IX). The compound was recrystallized from acetone–petroleum ether (b.p. 90–95°) to yield 0.573 g. of the desired lactone IX, m.p. 212–213°.

An analytical sample had a m.p. of 212–213°; $[\alpha]_D^{25}$ +76° (*c*, 1.07 chloroform); $\lambda_{max}^{CH_3OH}$ 239 mμ (ε 18,200); $\lambda_{max}^{CHCl_3}$ 5.66 μ, 6.00 μ, 6.17 μ, 8.53 μ.

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 76.40; H, 8.34. Found: C, 76.32; H, 8.46.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

Sulfur Substitution Compounds of Amino Sugars. I 1-Thio-D-glucosamine[1]

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We have attempted to devise synthetic methods for the preparation of a new class of carbohydrates, the 2-amino-2-deoxy-1-thioaldoses, of which 1-thio-D-glucosamine hydrochloride (XI) is a prototype. Various preliminary attempts to prepare XI, unsuccessful, but leading to crystalline derivatives of XI, as well as a successful preparation of XI, are described.

Besides a small number of alkyl thioglycosides and thioacetals of D-glucosamine (2-amino-2-deoxy-D-glucose)[2] no sulfur-containing substitution products of aminosugars seem to be known. Only very recently Christensen and Goodman[3] described a potential starting material for the synthesis of 2,3-, and 3,2-thioamino sugars. On the other hand thio- derivatives of nitrogen-free carbohydrates are well known and readily available. About fifty years ago Schneider and co-workers[4] published a series of papers dealing with the synthesis and reactions of 1-thio-D-glucose, the most accessible of the various thioglucose isomers. However, crystalline unsubstituted thioglucose derivatives have yet to be described, despite continued interest in these substances.[4,5] Our own interest in this field has directed our studies toward the synthesis of 1-thio-D-glucosamine and its disulfide, prototypical of two new classes of *S,N*-containing carbohydrates, making use of several of the polyacetyl-α-D-glucosaminyl halide derivatives already described in the literature. Of the methods known for introducing a mercapto group we have chosen the reaction of such halogeno compounds with potassium thioacetate, with the hope of obtaining easily hydrolyzable *S*-acetyl derivatives[6] as precursors.

Reaction of 3,4,6-tri-*O*-acetyl-*N*-acetyl-α-D-glucosaminyl chloride[7] with potassium thioacetate gave the expected 3,4,6-tri-*O*-acetyl-*N*-acetyl-*S*-acetyl-1-thio-β-D-glucosamine (I) in 80% yield. Hydrolysis of the latter with ammonia in methanol without exclusion of air led, with simultaneous autooxidation, to di(*N*-acetyl-β-D-glucosaminyl) disulfide (II). Attempts to hydrolyze I by the Zemplen method failed to give an *O*-acetyl-free product. Reacetylation of II gave the corresponding acetate, di(3,4,6-tri-*O*-acetyl-*N*-acetyl-β-D-glucosaminyl) disulfide (III). Reduction of III under acetylating conditions[8] regenerated the starting material I. The course of these reactions was readily followed with infrared spectra, which showed the presence or absence of *O*-, *S*-, and *N*-acetyl groups unambiguously.

As it has proved impossible to hydrolyze the *N*-acetyl group in I, II, and III without affecting the glycosidic center of these compounds, it became obvious that a more readily removable nitrogen substituent would be required before unsubstituted 1-thio-D-glucosamine could be obtained. A compound promising in this connection appeared to us to be the recently described[9] 3,4,6-tri-*O*-acetyl-*N*-

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(2) H. H. Baer, *Fortschr. chem. Forsch.*, 3, 859 (1958); H. Weidmann and H. K. Zimmerman, *Ann. Chem. Liebigs*, 628, 255 (1959).

(3) J. E. Christensen and L. Goodman, *J. Am. Chem. Soc.*, 82, 4738 (1960).

(4) Reviewed by A. L. Raymond in *Advances in Carbohydrate Chem.*, *I*, 129 (1945).

(5) See, for instance, F. B. Fraser, L. N. Owen, and G. Shaw, *Biochem. J.*, 41, 328 (1947); V. Prey and F. Grundschober, *Monatsh. Chem.*, 91, 358 (1960); M. Cerny, F. Vrkoc, and F. Stanek, *Coll. Czech. Chem. Comm.*, 24, 64 (1959); M. Cerny and F. Pacak, *Coll. Czech. Chem. Comm.*, 24, 2566 (1959).

(6) M. Gehrke and W. Kohler, *Ber.*, 64, 2696 (1931).

(7) D. H. Leaback and P. G. Walker, *J. Chem. Soc.*, 4754 (1957).

(8) F. Wrede, *Z. physiol. Chem.*, 119, 46 (1922).

(9) L. Zervas and S. Konstas, *Chem. Ber.*, 93, 435 (1960).