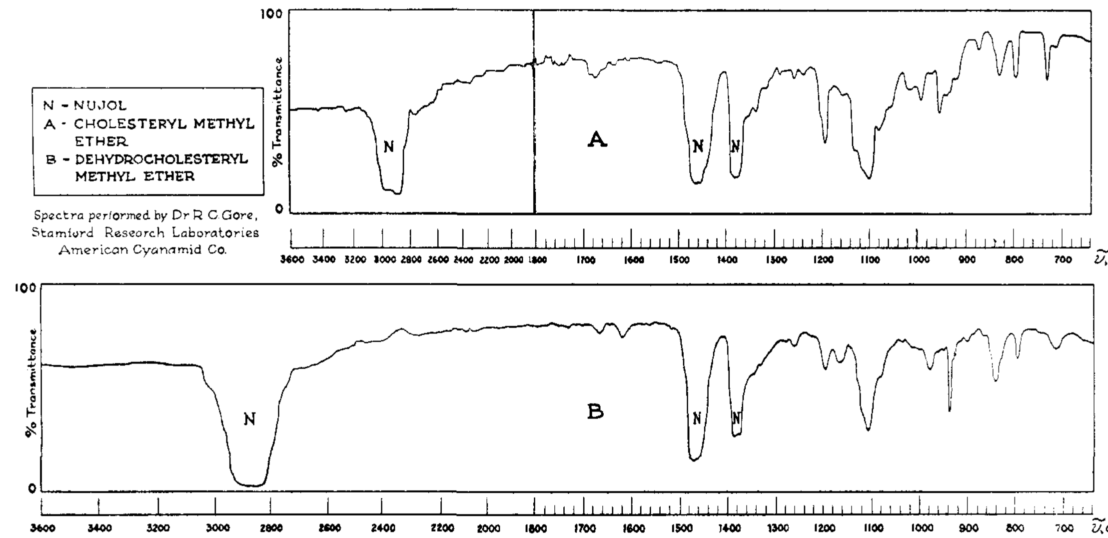

Fig. 1.—Infrared spectra (Nujol mull).

$\Delta^{5,7}$- and $\Delta^{4,6}$-ethers. No further experiment to isolate pure $\Delta^{4,6}$-ether was attempted.

Experimental

7-Dehydrocholesteryl Methyl Ether.—A mixture of 8.0 g. (0.02 *M*) of cholesteryl methyl ether and 4.28 g. (0.024 *M*) of NBS in 100 ml. of petroleum ether, b.p. 64–66° (purified with concd. sulfuric acid and potassium permanganate) was refluxed and irradiated for 3.5 minutes by the heat and light of 2 photospot lamps (type RSP-2, General Electric Co.). Eight ml. of *s*-collidine was then added to the refluxing mixture, which was cooled and filtered. The filtrate was evaporated *in vacuo* (nitrogen atmosphere); the distillation temperature was maintained at room temperature and below. Eighty ml. of xylene was added to the residue. The mixture was refluxed for 12 minutes (nitrogen atmosphere), cooled, and the solid was separated by filtration. The filtrate on evaporation *in vacuo* (nitrogen atmosphere) gave an oily residue which crystallized on treatment with acetone; wt. 5.06 g., m.p. 83–109°; $\lambda^{1\%\ \mathrm{CA}}_{\mathrm{max.}}$[6] 240, 272, 282 and 294 mμ; ϵ_{240} = 9050, ϵ_{282} = 6360. Six recrystallizations to constant melting point from acetone gave the pure $\Delta^{5,7}$-ether, wt. 0.76 g., m.p. 123–125°; $\lambda^{1\%\ \mathrm{CA}}_{\mathrm{max.}}$ 272, 282 and 294 mμ; ϵ_{272} = 9900, ϵ_{282} = 10600, ϵ_{294} = 5950; $[\alpha]^{27}\mathrm{D}$ −104.3° (19.55 mg. in 2 ml. of chloroform, 1 dcm. semi-micro tube, gave $\alpha^{27}\mathrm{D}$ −1.02°), $[M]\mathrm{D}$ −415.

Anal.[7] Calcd. for $C_{28}H_{46}O$ (398.65): C, 84.35; H, 11.63; OCH_3, 7.78. Found: C, 84.19; H, 11.80; OCH_3, 7.99.

The material in the mother liquors was triangularly recrystallized from acetone and acetone–methanol, and gave an additional 0.26 g. of the $\Delta^{5,7}$-ether, m.p. 122–124°, and two fractions consisting of a mixture of $\Delta^{4,6}$- and $\Delta^{5,7}$-ethers, m.p. 86.5–88.5° and 86–88°, $\lambda^{1\%\ \mathrm{CA}}_{\mathrm{max.}}$ 239, 271.5, 282 and 293–294 mμ.

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[6] Solvent was 1% chloroform–absolute alcohol; the substance was dissolved in 1 ml. of chloroform and rapidly diluted to 100 ml. with absolute alcohol.

[7] We are indebted to Messrs. Louis M. Brancone and Samuel M. Modes for the microanalytical data.

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The Reduction of Estrone and Estrogen Esters

BY JOHN H. BIEL

Difficultly hydrolyzable esters of β-estradiol have been shown to possess a greater clinical usefulness than β-estradiol itself.[1] It was desirable therefore to develop a process for the reduction of highly branched esters of estrone to the corresponding β-estradiol 3-esters. Various hydrogenation procedures for estrone and its esters have been described in the chemical and patent literature. Thus Miescher and Scholz[2] were able to reduce aliphatic esters of estrone to estradiol 3-esters by catalytic hydrogenation in ethyl acetate. Marker and Rohrmann[3] converted the trimethylacetate and *t*-butylacetate of estrone to the corresponding β-estradiol derivatives with Adams catalyst in neutral medium at atmospheric pressure and room temperature. Estrone has also been reduced by means of lithium aluminum hydride[4] to β-estradiol and by aluminum isopropoxide[5] to a mixture of β-estradiol and α-estradiol.

While estrone esters could be reduced successfully by catalytic hydrogenation, the method was impracticable for our purpose. Both lithium aluminum hydride and calcium hydride cleaved the esters to β-estradiol and estrone, respectively, during hydrogenation even under mild conditions (see Experimental). Dirscherl[6] reported a similar cleavage of estrone esters during catalytic reduction.

The use of sodium borohydride for the reduction of aldehydes, ketones and acid chlorides was first reported by Chaikin and Brown.[7] We found that this reagent afforded a simple and convenient method for obtaining β-estradiol and two of its highly branched aliphatic esters in excellent yield and high purity from estrone and the corresponding estrone esters. Estrone esters where the acid moiety did not contain a highly branched side chain such as the acetate, propionate, butyrate and benzoate yielded either β-estradiol or a mixture of prod-

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[1] W. Brown and J. T. Bradbury, *J. Clin. Endocrinol.*, **8**, 612 (1948).

[2] K. Miescher and C. Scholz, U. S. Patent 2,156,599 (1939).

[3] R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 1922 (1939).

[4] A. C. Ott and M. F. Murray, Abstracts of the 113th Meeting of the American Chemical Society, April, 1948.

[5] R. E. Marker and E. Rohrmann, THIS JOURNAL, **60**, 2927 (1938).

[6] W. Dirscherl, *Z. physiol. Chem.*, **239**, 53 (1936).

[7] S. W. Chaikin and W. G. Brown, THIS JOURNAL, **71**, 122 (1949).

ucts from which only β-estradiol could be isolated in pure form.

Experimental

The estrone esters were prepared in excellent yield by the method of Miescher and Scholz.[8]

β-Estradiol.—A mixture of 400 mg. of estrone, 0.2 cc. of 20% sodium hydroxide solution and 20 cc. of methanol was added to a solution of 150 mg. of sodium borohydride in 20 cc. of methanol. After the evolution of hydrogen had ceased, the reaction mixture was poured into 30 cc. of water and neutralized with dilute hydrochloric acid. The precipitate was filtered, washed with water and immediately recrystallized from aqueous methanol; yield 360 mg. (90%), m.p. 172–174°. A mixed m.p. with an authentic sample of β-estradiol showed no depression.

The Reduction of Estrone Trimethylacetate. A. By Lithium Aluminum Hydride.—To 900 mg. (0.0025 mole) of estrone trimethyl acetate dissolved in 100 cc. of anhydrous ether was added with stirring 200 mg. (0.0052 mole) of lithium aluminum hydride in 15 cc. of anhydrous ether. The mixture was stirred for another hour, cooled and 10 cc. of water added. A precipitate formed, which was dissolved by the addition of 10 cc. of ice-cold 10% sulfuric acid.[9] The aqueous layer was separated and the ether layer concentrated to dryness *in vacuo*. The white, crystalline residue was suspended in 50% aqueous methanol and filtered; yield 700 mg. (100%), m.p. 175–177°. A mixed m.p. with an authentic sample of β-estradiol showed no depression.

When stoichiometric amounts of lithium aluminum hydride were used 85% of the estrone trimethylacetate was recovered unchanged. The remainder could not be purified further, but gave a very strong positive test for free phenolic groups with diazotized sulfanilic acid.

B. By Calcium Hydride.—A mixture of 900 mg. (0.0025 mole) of estrone trimethylacetate, 210 mg. (0.0050 mole) of calcium hydride and 75 cc. of 97% aqueous methanol was allowed to stand until the evolution of hydrogen had ceased. The filtrate was added to an equal volume of water, the precipitate filtered and 250 mg. of material collected, m.p. 156–160°. After recrystallization from methanol 200 mg. of the product was recovered; m.p. 165.5–166.5°. A mixed m.p. with an authentic sample of estrone trimethylacetate showed no depression. The aqueous methanol filtrate was chilled and 250 mg. of a solid collected, m.p. 247–255°. After recrystallization from methanol the material melted at 254–256°. A mixed m.p. with an authentic sample of estrone showed no depression.

β-Estradiol 3-Trimethylacetate.—To 250 mg. (0.0062 mole) of sodium borohydride in 10 cc. of methanol was added 900 mg. of estrone trimethylacetate dissolved in 10 cc. of hot methanol. After hydrogen evolution had ceased (*ca.* 30 minutes), 20 cc. of water was added slowly with stirring, the precipitate collected by filtration, washed with 50% methanol and immediately recrystallized from aqueous methanol; yield 600 mg., m.p. 179–181°. To the boiling filtrate water was added to a point just short of turbidity. On cooling, 200 mg. of product was obtained after filtration, m.p. 178–180°. The two fractions were combined; yield 800 mg. (89%); a mixed m.p. with a sample of β-estradiol 3-trimethylacetate obtained from the catalytic hydrogenation of estrone trimethylacetate[9] gave no depression. A test with diazotized sulfanilic acid showed the absence of free phenolic groups.

β-Estradiol 3-*t*-Butylacetate.—The procedure was identical with the one described for the preparation of the trimethylacetate. From 500 mg. of estrone ester there was obtained 350 mg. (70%) of the β-estradiol 3-ester, m.p. 127–129°. A mixed m.p. with a sample obtained from the catalytic hydrogenation of estrone *t*-butylacetate[9] gave no depression.

The Reduction of Estrone Acetate with Sodium Borohydride.—A solution of 500 mg. of estrone acetate in 10 cc. of methanol was added to 150 mg. of sodium borohydride in 10 cc. of methanol and the reaction mixture allowed to stand for 1 hour at room temperature, at which time no more hydrogen was evolved. Thirty cc. of water was then added slowly to the cooled solution and the resulting precipitate filtered and washed with cold 50% methanol; yield 400 mg., m.p. 170–175°. After recrystallization from aqueous methanol 350 mg. of a crystalline precipitate was obtained, m.p. 174–175°. A mixed m.p. with an authentic sample of β-estradiol showed no depression. The product coupled readily with diazotized sulfanilic acid showing the presence of a free phenolic group.

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[8] K. Miescher and C. Scholz, *Helv. Chim. Acta*, **20**, 263 (1937).

[9] An aqueous alcoholic acid solution of β-estradiol trimethylacetate showed no signs of hydrolysis after standing at room temperature for several hours.

The Oxidation of Glucose and Related Compounds with Oxides of Nitrogen[1]

BY ED. F. DEGERING[2] AND ROBERT A. BERNTSEN[3]

Saccharic acid has been prepared in moderate (40–46%)[4] yields by the oxidation of glucose with nitric acid.[4,5,6] The importance of saccharic acid[4] and the fact that acids have been obtained by the nitrogen tetroxide oxidation of certain compounds containing a primary hydroxyl group, such as cellulose,[7,8] starch[9] and galactose,[10] prompted this experimental work.

The accompanying experimental work shows that glucose when oxidized by nitrogen tetroxide at 35° in the presence of potassium carbonate gives potassium acid saccharate in 50% yield. Gluconic acid can be oxidized in a nitrogen tetroxide medium to give the same substance in 42% yield. Oxalic acid was not attacked. Potassium acid saccharate could be recovered in 85% yield after treatment with nitrogen tetroxide, but since the salt is relatively soluble in water the loss appears to be mechanical in nature.

Experimental

Materials.—Anhydrous dextrose (C.P., Mallinckrodt) dried in a vacuum desiccator over phosphorus pentoxide or glucose (American Maize Products Co.) dried in a vacuum desiccator at 70° and 10 mm. pressure was used. Nitrogen tetroxide (Solvay Process Company) was dried over phosphorus pentoxide before use.

Oxidation of Glucose in an Inert Medium.—Glucose (0.1 mole) was added to 100 ml. of the inert medium (carbon tetrachloride, petroleum ether or toluene) in a 500-ml., round-bottom three-neck flask which contained a Dry Ice finger-type condenser and an efficient stirrer. The nitrogen tetroxide (0.09 or 0.17 mole) was added through a graduated ice water-cooled West condenser with attached stopcock and ground-glass joint. The experiments were carried out in a constant temperature water-bath at 25 ± 1° for a period of time from 1 to 96 hours. After the period of oxidation, the excess nitrogen tetroxide was blown out and the inert medium decanted off. The oxidation product was washed once with ether and then dissolved in water and the *p*H of the solution was adjusted to 9 using potassium carbonate, potassium hydroxide or both. After hydrolysis of the lactone ring, enough acetic acid was added to adjust the *p*H to 4. The resulting potassium acid saccharate was filtered off, air-dried, and weighed. The yield varied from 10 to 44%.

In a similar manner except for the presence of 0.1 g. of aluminum chloride, cobalt oxide, copper acetate, copper

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[1] From the Ph.D. Thesis of Robert A. Berntsen, Purdue University.

[2] Miner Laboratories, 9 South Clinton, Chicago 6, Ill.

[3] Augustana College, Rock Island, Ill.

[4] C. L. Mehltretter, U. S. Patent 2,436,659 (Feb. 24, 1948).

[5] A. A. Dietz, Ph.D. Thesis, Purdue University, 1941.

[6] J. W. Mench, Ph.D. Thesis, Purdue University, 1944.

[7] C. Unruh and W. O. Kenyon, THIS JOURNAL, **64**, 127 (1942).

[8] E. C. Yackel and W. O. Kenyon, *ibid.*, **64**, 121 (1942).

[9] J. W. Mench with Ed. F. Degering, *Proc. Ind. Acad. Sci.*, **55**, 69 (1946).

[10] K. Maurer and G. Drefahl, *Ber.*, **75**, 1489 (1942).