TABLE I

MOLECULAR ROTATIONS OF 16-KETOSTEROIDS

Parent compound	$[\alpha]_D$	M_D	16-Keto derivative $[\alpha]_D$	16-Keto derivative M_D	ΔM_D[a]
Androstan-3β-ol	0° (chf)[b]	0	−180° (diox)[c]	−523	523
$\Delta^{1,3,5(10)}$-Estratrien-3-ol (desoxyestrone)	+88° (alc)[d]	+226	−87° (alc)[e]	−236	462
$\Delta^{1,3,5(10)}$-Estratriene-3,17β-diol (estradiol-17β)	+80° (diox)[e]	+218	−102° (alc)[f]	−293	511
Δ^4-Androsten-17β-ol-3-one (testosterone)	+109° (alc)[g],(chf)[i]	+314	−52° (chf)[h]	−158	472
Δ^4-Androsten-17β-ol-3-one acetate	+96° (chf)[i]	+317	−29° (chf)[h]	−100	417

[a] No correction for the difference in solvent has been made. [b] L. Ruzicka, V. Prelog and T. Meister, *Helv. Chim. Acta*, **28**, 1651 (1945). [c] M. N. Huffman and M. H. Lott, THIS JOURNAL, **73**, 878 (1951). [d] A. Butenandt and U. Westphal, *Z. physiol. Chem.*, **223**, 147 (1934). [e] A. Girard, G. Sandulesco and A. Fridenson, *Compt. rend. soc. de biol.*, **112**, 964 (1933). [f] M. N. Huffman, THIS JOURNAL, **64**, 2235 (1942). [g] K. David, E. Dingemanse, J. Freud and E. Laqueur, *Z. physiol. Chem.*, **233**, 281 (1935). [h] Experimental part. [i] F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).

crystallized from aqueous methanol and melted after two recrystallizations from acetone–ether at 152–158°; $[\alpha]^{22}D$ −52° in chloroform. Acetylation produced IV with m.p. 195–199° from ether; $[\alpha]^{25}D$ −29° in chloroform. The molecular rotations (M_D) for III and IV indicate that the molecular rotatory contribution[5] of the 16-keto group is strongly negative (*e.g.*, for cpd. III–472) which is in concordance with values calculated for other known 16-ketosteroids (Table I). If the neighboring 17β-hydroxy group is acetylated, the contribution appears to be of a somewhat lower magnitude (for cpd. IV–417). Likewise a smaller rotatory contribution for the introduction of a ketone group vicinal to an acetate instead of a hydroxyl group can be observed on other positions of the molecule (*cf.*, *e.g.*, in the cholanic acid series the 11,12β-, 12,11α-, and 12,11β-ketols and ketol acetates).

Experimental[6]

One-hundred mg. of crude 16-isonitroso-Δ^4-androstene-3,17-dione (II) with m.p. 220–226° was reduced in an aqueous acetic acid solution with zinc dust as described.[2] Seventy-five mg. of a neutral extract was obtained. One-fifth of this quantity was applied along a line 7 cm. from the narrow end of a strip of washed filter paper (17 × 57 cm.) which was previously immersed in a propylene glycol–methanol (1:1) solution and immediately blotted. Five such sheets were chromatographed for 5 hours at 25° in tanks with propylene glycol saturated toluene according to the technique of Burton, Zaffaroni and Keutmann.[7] In this period the main product moved to a zone extending from 22 to 28 cm. from the line of application as evidenced by its absorption of ultraviolet light of 2537Å. due to the Δ^4-3-keto structure. It gave the pink color with the triphenyltetrazolium ketol reagent[7] and a gray-purple with the *m*-dinitrobenzene Zimmermann reagent.[8] Three other zones of slower moving substances present in low concentrations were noted in addition; one of these showed both the ultraviolet absorption and the tetrazolium reaction while the other demonstrated only one each of the two tests. After drying the papers at room temperature in an air draft, the areas containing the main product were exhaustively extracted with acetone. Sixty-eight mg. of a residue was obtained and dissolved in 10 drops of methanol. Upon addition of one drop of water a slight turbidity appeared which after standing overnight at 4° had vanished. A minute amount of crystals was then detected. Gradual additions of more water led to an abundant development of these crystals. The water concentration was eventually raised to approximately 50% at which point the supernatant no longer clarified on standing. The crystals were filtered, washed and dried *in vacuo* producing 54 mg. of III with m.p. 149–157°. Recrystallization from acetone–ether resulted in 43 mg. of Δ^4-androsten-17β-ol-3,16-dione (III) with m.p. 152–158°. The m.p. remained constant after a further crystallization and the product showed $[\alpha]^{22}D$ −52 ± 2° (1.2% in chloroform); light absorption at 2405 Å. with ϵ_{max} 16,600 in 95% ethanol and near 1667 and 1618 cm.$^{-1}$ (C=O, α,β-unsaturated), 3478 (O–H), 1748 (five ring C=O), 1088, 1070, 1055, 1033 cm.$^{-1}$ (some fingerprint bands).

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.45; H, 8.67. Found: C, 75.47; H, 8.88.

Acetylation.—1.6 mg. of III was dissolved in 0.2 ml. of pyridine and allowed to stand for 17 hours at room temperature with 0.12 ml. of acetic anhydride. After the usual processing 2.0 mg. of a neutral residue was obtained. This was crystallized once from an ether–pentane mixture to yield 1.5 mg. of compound IV with a m.p. 195–199°. A recrystallization from ether did not change the m.p., and the product showed $[\alpha]^{25}D$ −29 ± 3° (0.45% in chloroform); infrared absorption near 1751 (five ring C=O), 1730 (acetate C=O), 1661 and 1618 (C=O, α,β-unsaturated), 1238 and 1229 (acetate C–O), 1089, 1072, 1053, 1003 cm.$^{-1}$ (some fingerprint bands).

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(5) *Cf.* L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd edition, Reinhold Publ. Corp., New York, N. Y., 1949, p. 204.

(6) Melting points are corrected; infrared data established on samples in the solid state with a Perkin–Elmer 12 C spectrometer by Dr. H. Rosenkrantz and Mr. P. Skogstrom; microanalysis by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(7) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

(8) W. Zimmermann, *Vitamine und Hormone*, **5**, 1 (1944).

3-Aminophenoxathiin

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As an extension of the work recently reported[3] describing the preparation of certain new amino and nitro derivatives of phenoxathiin by direct nuclear substitution reactions, the synthesis of 3-aminophenoxathiin has now also been accomplished. The useful rearrangement technique involving amination of *o*-halogenated ethers[4] was found to be ideally suited to the preparation of this new amino derivative. Thus, the reaction of 4-iodophenoxathiin with sodamide in liquid ammonia gave a 31% yield of 3-aminophenoxathiin. The 4-iodophenoxathiin was prepared by the reaction of 4-phenoxathiinyllithium with iodine.

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(2) This paper comprises part of a thesis submitted by N. W. Burske in partial fulfillment of the requirements for the degree of Master of Science at Xavier University.

(3) J. F. Nobis, A. J. Blardinelli and D. J. Blaney, THIS JOURNAL, **75**, 3384 (1953).

(4) H. Gilman and S. Avakian, *ibid.*, **67**, 349 (1945); see also the list of references in R. A. Benkeser and W. E. Buting, *ibid.*, **74**, 3011 (1952).

Although the melting point of this new aminophenoxathiin (81.5–83°) was different than that of either the known[3] 2-amino (93–95°) or the 4-amino[5] (b.p. 209° (5 mm.)) derivatives and a similar reaction in the related heterocycle dibenzothiophene had given rearrangement,[6] additional evidence of the structure was obtained. The new amine was converted to an iodophenoxathiin (m.p. 70–72°) by diazotization and subsequent reaction with potassium iodide. The iodo compound was shown to be different from either the 2-iodophenoxathiin (m.p. 92–93°) or the 4-iodophenoxathiin (m.p. 42.5–43°) by the method of mixed melting points. The 2-iodophenoxathiin had been prepared from 2-aminophenoxathiin through the diazonium salt. It should be noted that both the 2-amino and 3-amino derivatives can be diazotized only with difficulty and special conditions are necessary. Thus, a longer reaction period with sodium nitrite is required and temperatures in the range of 5–10° must be used.

An attempt was made to prepare the desired 3-aminophenoxathiin by nitration of 2-acetaminophenoxathiin followed by deacetylation, diazotization, deamination and reduction of the resulting nitro compound. However, the identity of the nitration product could not be established since a mixture of products resulted.

Experimental Part

4-Iodophenoxathiin.—4-Phenoxathiinyllithium was prepared in essential accordance with the procedure of Gilman, Van Ess, Willis and Stuckwisch[5] from 0.47 mole of *n*-butyllithium and 60 g. (0.4 mole) of phenoxathiin in 600 ml. of anhydrous ether over a period of 20 hours. At the end of this time, the reaction mixture was cooled in an ice-bath and 126.5 g. (0.5 mole) of powdered iodine added in small portions. When the addition was complete, the reaction mixture was allowed to stir for one-half hour or until Color Test I[7] was negative. The excess iodine was removed with bisulfite; the layers were separated; and the ether layer dried over sodium sulfate. After removal of the ether, the heavy viscous oil was distilled under reduced pressure. There was obtained 35 g. of product boiling at 135–170° (2 mm.). Recrystallization from petroleum ether (b.p. 60–90°) gave 27.2 g. (21%) of pure 4-iodophenoxathiin melting at 42.5–43°.

Anal. Calcd. for $C_{12}H_7OIS$: I, 38.92; S, 9.83. Found: I, 39.41; S, 9.78.

There was also obtained a higher boiling fraction (170–190° (1 mm.)) believed to be a mixture of 4-iodophenoxathiin and 4,6-diiodophenoxathiin but separation could not be accomplished.

3-Aminophenoxathiin.—Sodamide was prepared according to directions of Vaughn, Vogt and Nieuwland[8] from 8.1 g. (0.35 g. atom) of sodium and 0.5 g. of ferric nitrate in 300 ml. of liquid ammonia. To this solution was added 38.5 (0.1 mole) of 4-iodophenoxathiin in 100 cc. of benzene over a 20-minute period. The excess sodamide was decomposed with ammonium chloride and the ammonia removed by evaporation. The crude amine that remained was dissolved in benzene and separated from the inorganic salts by filtration. Dry hydrogen chloride was admitted to the benzene solution to precipitate the amine hydrochloride. Treatment of this solid with ammonium hydroxide gave a dark oil that was purified with carbon in a hot 70% methanol–water mixture. After filtering and cooling, there was obtained 8 g. (31%) of product melting at 81.5–83°. A mixed melting point with 2-aminophenoxathiin was depressed.

Anal. Calcd. for $C_{12}H_9ONS$: S, 14.9. Found: S, 14.7.

3-Acetaminophenoxathiin.—One g. (0.005 mole) of crude 3-aminophenoxathiin in 25 cc. of benzene containing 0.5 g. (0.005 mole) of acetic anhydride was refluxed for three hours. At the end of this time the benzene was removed and the crude oil crystallized from methanol to give 0.3 g. (25%) of 3-acetaminophenoxathiin melting at 181–182.5°.

Anal. Calcd. for $C_{14}H_{11}ON_2S$: S, 12.46. Found: S, 12.85.

3-Iodophenoxathiin.—A mixture of 2 g. (0.009 mole) of 3-aminophenoxathiin, 10 ml. of concentrated sulfuric acid (sp. gr. 1.81) and 200 ml. of water was heated to boiling and allowed to cool slowly to room temperature. The reaction flask was then cooled to 5° and 0.7 g. (0.01 mole) of sodium nitrite in 20 ml. of water added over a ten-minute interval. The temperature of the reaction mixture was held between 5–10° during the addition and for another hour after addition was complete. The diazonium salt was then treated with 0.1 g. of urea followed by the addition of 1.5 g. (0.01 mole) of potassium iodide in 20 ml. of water. The bright red solution was warmed to 70° to ensure completion of the reaction. The resulting mixture was extracted with ether and the extracts washed with 5% sodium bisulfite solution. Removal of the ether gave a brown oil that was crystallized from methanol. There was obtained 0.45 g. (15%) of 3-iodophenoxathiin melting at 70–72°. Mixed melting points with both the 2-iodo- and 4-iodophenoxathiin were depressed.

Anal. Calcd. for $C_{12}H_7OIS$: S, 9.83. Found: S, 10.12.

2-Iodophenoxathiin.—2-Aminophenoxathiin[3] was diazotized in the same manner as described for 3-aminophenoxathiin. Thus, from 3.5 g. (0.016 mole) of the 2-amino compound, 12 ml. of sulfuric acid, 300 ml. of water and 1.2 (0.017 mole) of sodium nitrite, there was obtained 0.55 g. (10.4%) of 2-iodophenoxathiin melting at 92–94° after recrystallization from dilute methanol.

Anal. Calcd. for $C_{12}H_7OIS$: S, 9.83. Found: S, 9.53.

Nitration of 2-Acetaminophenoxathiin.—Nitration of 4 g. (0.016 mole) of 2-acetaminophenoxathiin[3] in 50 ml. of acetic anhydride with 2 ml. of concd. nitric acid (sp. gr. 1.42) at −4° over a 20-minute period gave a crude yellow product which was recrystallized from methanol. There was obtained 0.1 g. of material melting at 174–176.5°. The analytical results indicated that this material might be 2-acetamino-3,8-dinitrophenoxathiin. None of the desired 2-acetamino-3-nitrophenoxathiin could be isolated.

Anal. Calcd. for $C_{14}H_9O_6N_2S$: S, 9.23. Found: S, 9.48.

Nitration of 2-Acetaminophenoxathiin 5-Dioxide.—Attempts to nitrate 2-acetaminophenoxathiin 5-dioxide[3] under a variety of conditions gave only mixtures of products from which no pure material could be isolated.

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(5) H. Gilman, J. P. Van Ess, H. B. Willis and C. G. Stuckwisch, THIS JOURNAL, **62**, 2606 (1940).

(6) H. Gilman and J. F. Nobis, *ibid.*, **67**, 1479 (1945).

(7) H. Gilman and F. Schultz, *ibid.*, **47**, 2002 (1925).

(8) T. H. Vaughn, R. R. Vogt and J. A. Nieuwland, *ibid.*, **56**, 2120 (1934).

Crystalline Brucine Salts of Oligogalacturonides[1]

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During the hydrolysis of polygalacturonic acid with dilute acid or the enzyme polygalacturonase, a series of oligogalacturonic acids is produced. Jermyn and Tomkins, using paper chromatography,[2] tentatively identified galacturonic acid after enzymic hydrolysis. Galacturonic, di-, tri- and tetragalacturonic acids have been obtained in pure form[3-4] and we have succeeded in crystallizing their brucine

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(2) M. A. Jermyn and R. G. Tomkins, *Biochem. J.*, **47**, 437 (1950).

(3) (a) H. J. Phaff and B. S. Luh, *Arch. Biochem. and Biophys.*, **33**, 212 (1951); (b) H. Altermatt and H. Deuel, *Helv. Chim. Acta*, **35**, 1422 (1952); **36**, 340 (1952).

(4) J. K. N. Jones and W. W. Reid, *Chem. and Ind.*, 303 (1953).