The ethereal filtrate from the trituration was concentrated and 18.5 g. (46%) of a thick yellow water-soluble oil was obtained. A sample was taken up in ether, decolorized with charcoal and taken to dryness under high vacuum.

Anal. Calcd. for $C_{24}H_{45}N_9O_3$ (II): C, 56.77; H, 8.93; N, 24.81. Found: C, 56.67; H, 8.96; N, 24.83.

2,4,6-Tri-(γ-morpholinopropyl) Cyanurate Trihydrochloride (III).—The procedure of Spielman, Close and Wilk[3] for non-basic cyanurates was modified for this preparation. Sodium hydride (2.52 g., 0.105 mole) was added to γ-morpholinopropanol (21.75 g., 0.15 mole) in 25 cc. of dry benzene and the mixture was refluxed overnight. To this stirred mixture, after cooling in ice, was added dropwise cyanuric chloride (6.072 g., 0.033 mole) dissolved in a minimum amount of dry benzene. The mixture was then refluxed for one hour, filtered and concentrated. The residual oil was submitted to distillation and the low-boiling material was removed at 64° (0.4 mm.). The distillation was stopped and the undistilled oil was dissolved in ether and filtered. The trihydrochloride was made by treatment with excess ethereal HCl. It was recrystallized by dissolving in dry methanol and adding an equal volume of dry isopropyl alcohol and cooling. There was obtained 15 g. (73%) of a white powder, m.p. 272–275° dec.

Anal. Calcd. for $C_{24}H_{42}N_6O_6 \cdot 3HCl$ (III): C, 46.48; H, 7.31; N, 13.55. Found: C, 45.98; H, 7.58; N, 13.01.

Tri-(β-dimethylaminoethyl) Cyanurate Trimethiodide (IV).—This compound was prepared in a manner similar to that employed in the preparation of III. In this case 4.8 g. (0.20 mole) of sodium hydride, 18.7 g. (0.21 mole) of β-dimethylaminoethanol and 12.14 g. (0.066 mole) of cyanuric chloride was used. The mixture was refluxed 1.5 hours instead of overnight. After removal of the solvent the dark brown semi-solid residue was stirred well with ether. After filtering, the ether solution was concentrated. The resulting thick oil was taken up in absolute alcohol and excess methyl iodide was added. Precipitation of the quaternary salt began almost immediately. After standing overnight and filtering, a crude yellow solid was obtained. After repeated recrystallization by suspending the solid in boiling ethanol or methanol and adding water dropwise until solution occurred, a 10% yield (based on cyanuric chloride) of colorless leaflets, m.p. 214–215° dec., was obtained.

Anal. Calcd. for $C_{18}H_{30}N_6O_3 \cdot 3CH_3I$ (IV): C, 28.16; H, 5.11; N, 10.93. Found: C, 27.92; H, 5.20; N, 10.73.

Acknowledgment.—The analyses were carried out by E. F. Shelberg, Chief Microanalyst, and his staff.

(3) M. A. Spielman, W. J. Close and I. J. Wilk, THIS JOURNAL, **73**, 1775 (1951).

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N-{*p*-[(2,4-Diamino-6-pteridylmethyl)-tosylamino]-benzoyl}-L-glutamic Acid, a Pteroylglutamic Acid Analog

BY BARNEY J. MAGERLEIN AND DAVID I. WEISBLAT

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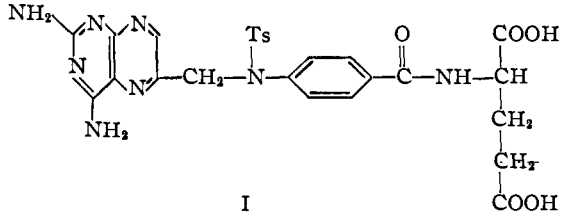
The condensation of 2,4,5,6-tetraminopyrimidine hydrochloride[1] with diethyl N-[N′-tosyl-N′-(3,3-diethoxy-2-ketopropyl)-*p*-aminobenzoyl]-L-glutamate[2] followed by saponification gave the pteridine, N-{*p*-[(2,4-diamino-6-pteridylmethyl)-tosylamino]-benzoyl}-L-glutamic acid (I).



I

This compound possessed neither folic acid activity nor antifolic acid activity when assayed with the test organism *S. faecalis R.*

(1) W. Traube, *Ber.*, **37**, 4544 (1904); M. F. Malette, E. C. Taylor, Jr., and C. K. Cain, THIS JOURNAL, **69**, 1814 (1947).

(2) D. I. Weisblat, B. J. Magerlein, D. R. Myers, A. R. Hanze, E. I. Fairburn and S. T. Rolfson, *ibid.*, **75**, 5983 (1953).

Acknowledgment.—The authors are indebted to Mr. L. Stubberfield and Mr. E. Stapert for the microbiological assays, to Dr. G. Pish and Mr. L. Scholten for the ultraviolet absorption data, and to Mr. W. A. Struck and associates for the microanalyses.

Experimental

N-{*p*-[(2,4-Diamino-6-pteridylmethyl)-tosylamino]-benzoyl}-L-glutamic Acid (I).—A solution of 4.77 g. of diethyl N-[N′-tosyl-N′-(3,3-diethoxy-2-ketopropyl)-*p*-aminobenzoyl]-L-glutamate,[2] 2.49 g. of 2,4,5,6-tetraminopyrimidine hydrochloride,[1] 1.5 ml. of concentrated hydrochloric acid and 5.0 ml. of 95% ethanol was heated under reflux for 2 hours. The ethanol was distilled under vacuum, the residue diluted with 25 ml. of water, and extracted with ethyl acetate. The *p*H of the aqueous solution was adjusted to 5.0, and, after cooling, the precipitate collected by centrifugation. It weighed 2.1 g. (45.7% yield).

A slurry of 0.50 g. of the crude pteridine and 0.15 g. of calcium hydroxide in 8 ml. of 0.5 *N* sodium hydroxide and 100 ml. of water was stirred at 25° for one hour and filtered. The filtrate was heated to 95° and again filtered. The *p*H of the hot filtrate was adjusted to 4.0. After 18 hours at 4° the yellow precipitate was separated by centrifugation. Following lyophilization this material was dried at 100° for 6 hours at 0.1 mm. pressure. It weighed 0.23 g.; $\lambda_{max.}^{0.1\ N\ NaOH}$ 229 mμ, $E_{1\ cm.}^{1\%}$ 470; 259 mμ, $E_{1\ cm.}^{1\%}$ 580; 270 mμ, $E_{1\ cm.}^{1\%}$ 130.

Anal. Calcd. for $C_{26}H_{26}N_8O_7S$: C, 52.52; H, 4.4; N, 18.85; S, 5.4. Found: C, 51.71; H, 4.47; N, 19.24; S, 5.15; ash, 1.23. Found (corrected for ash): C, 52.4; H, 4.5; N, 19.5; S, 5.2.

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Crystalline Δ⁴-Androsten-17β-ol-3,16-dione[1]

BY ANDRE S. MEYER AND MARJORIE C. LINDBERG

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Δ⁴-Androsten-17β-ol-3,16-dione (III) (16-ketotestosterone) was required for purposes of comparison. This substance was obtained as an oil in 1942 by Stodola and Kendall[2] through the nitrosation of the Δ⁴-androstene-3,17-dione 3-enol ethyl ether (I) to the 16-isonitroso derivative (II) and subsequent zinc–acetic acid reduction. Compound III yielded the crystalline monoacetate (IV) with a m.p. 194–195° from petroleum ether–acetone. An $[\alpha]^{25}_{4561}$ −56° in 95% ethanol was determined for IV synthesized by a second route.[3] Through their investigations on various 16,17-ketol steroids prepared by the nitrosation method[2] Huffman and Lott[4] were able to ascertain the steric arrangement of the hydroxyl group at position 17 as being β-oriented. Recently, in this Laboratory with the use of paper chromatography the resolution of compound III from the reduction mixture was accomplished. It

(1) This investigation was supported by a grant from G. D. Searle and Company, Chicago, Illinois.

(2) F. H. Stodola and E. C. Kendall, *J. Org. Chem.*, **7**, 336 (1942).

(3) F. H. Stodola and E. C. Kendall, *ibid.*, **6**, 837 (1941).

(4) M. N. Huffman and M. H. Lott, THIS JOURNAL, **71**, 719 (1949).

TABLE I

MOLECULAR ROTATIONS OF 16-KETOSTEROIDS

Parent compound	[α]D	MD	16-Keto derivative [α]D	16-Keto derivative MD	ΔMD[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

BY JOHN F. NOBIS[1] AND NORBERT W. BURSKE[2]

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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.

(1) Research Division, National Distillers Chemical Co., Cincinnati, Ohio.

(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).