sample[18] with the same physical constants, and the infrared spectra were identical.

Anal. Calcd. for $C_{27}H_{40}$: C, 88.94; H, 11.06. Found: C, 89.16; H, 11.20.

Treatment of Ergosteryl Acetate with HCl.—A solution (27 ml.) of ergosteryl acetate (6.1 g.) in chloroform, which was 0.06 *M* in HCl, was allowed to stand at room temperature. The ultraviolet spectrum was unchanged after four hours and 25 ml. of 0.47 *M* HCl was added. The spectrum of the $\Delta^{5,7}$-diene system had completely disappeared after six hours and was replaced by the spectrum (λ_{max} 250 mμ) of the B-isomers. The dark green solution was evaporated to dryness and the residue was crystallized from ether–methanol to yield 4.5 g. of the B-isomers as colorless needles melting unsharply at 100°, λ_{max} 250 mμ (ϵ 20,000). Fieser, *et al.*,[19] report λ_{max} 250 mμ (ϵ *ca.* 20,000) for ergosteryl-B_1 acetate. The mother liquor was chromatographed on alumina, but only traces of material were eluted by 5 and 10% benzene in petroleum ether which should have removed a hydrocarbon. An additional amount of the B-isomers was eluted by benzene.

(18) A forthcoming publication from this Laboratory will describe the preparation of anthracholestatetraene starting with 7-dehydrocholesterol.

(19) M. Fieser, W. E. Rosen and L. F. Fieser, THIS JOURNAL, **74**, 5397 (1952).

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

The Anthrasteroid Rearrangement. II. The Structural Proof of 1-Methyl-2,3,5,6-tetracarboxybenzene[1]

BY WILLIAM R. NES AND ERICH MOSETTIG

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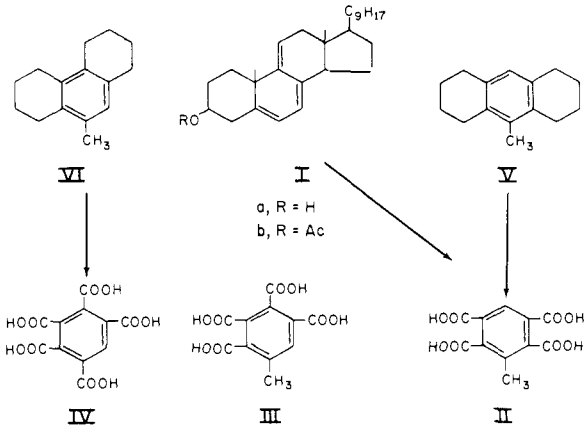
The acid which is obtained by the nitric acid oxidation of steroids containing two double bonds in ring B was found to be identical with the acid obtained from 9-methyl-*s*-octahydroanthracene. The acid is, therefore, 1-methyl-2,3,5,6-tetracarboxybenzene. The oxidation of 9-methyl-*s*-octahydrophenanthrene yielded pentacarboxybenzene. 9-Methyl-*s*-octahydrophenanthrene has been characterized by several new physical constants and has been prepared by a new route involving the previously unreported 9-hydroxymethyl-*s*-octahydrophenanthrene.

The structural proof of anthraergostapentaene[2] depends in part on the assignment of a correct formula to the methyltetracarboxybenzene of Inhoffen[3] and others.[4–6] In this paper the unequivocal preparation of this acid is described.

Although the use of nitric acid for degrading polynuclear hydrocarbons to an aromatic acid is usually applied to compounds containing one or more aromatic rings,[7] this technique has been successfully employed with non-aromatic compounds.[3–6] Thus it has been found that steroids containing two double bonds in ring B, *e.g.*, ergosterol, $\Delta^{6,8}$-coprostadienol and dehydroergosterol (Ia), yield an aromatic acid, while steroids in which ring B is not intact do not give this material. The methyl ester of this acid (prepared with diazomethane[3,6]) melts at 123–124°. Inhoffen[3] has shown that this acid must be either 1-methyl-2,3,5,6-tetracarboxybenzene (II) or 1-methyl-2,3,4,5-tetracarboxybenzene (III), since it forms a dianhydride and could be oxidized to pentacarboxybenzene (IV). For reasons which are not stated, later papers on the subject[5,6] ignore structure II and refer to this acid as III. Müller[6] actually misquotes Inhoffen on this subject. Feist[8] has reported the synthesis of II, but his methyl ester (m.p. 103–104°) was prepared with methanolic HCl which usually does not lead to a neutral ester,[9] and he gives no elemental analysis.



By oxidation of the appropriate *s*-octahydroanthracene and *s*-octahydrophenanthrene derivatives acids II and III, respectively, should be obtained. We have prepared 9-methyl-*s*-octahydroanthracene (V), essentially according to the directions of Badger, *et al.*,[10] and submitted it to oxidation with nitric acid. In order to obtain the reference acid, we oxidized dehydroergosteryl acetate (Ib). The products from the two oxidations were identical, and Inhoffen's acid is, therefore, 1-methyl-2,3,5,6-tetracarboxybenzene (II). It was hoped that in the analogous oxidation of 9-methyl-*s*-octahydrophenanthrene (VI) the methyl group would be unaffected and that the isomeric acid III would result. However, the only identifiable polycarboxylic acid was pentacarboxybenzene (IV). While both V and II have two *ortho* substituents, VI and III have only one substituent *ortho* to the methyl group, and this difference in steric hindrance may be

(1) A preliminary report of this work has been published in THIS JOURNAL, **75**, 2787 (1953). Presented in part at the National Meeting of the American Chemical Society, September 6–11, 1953.

(2) W. R. Nes and E. Mosettig, *ibid.*, **76**, 3182 (1954).

(3) H. H. Inhoffen, *Ann.*, **494**, 122 (1932).

(4) F. Reindel and K. Niederländer, *ibid.*, **482**, 264 (1930).

(5) A. Windaus and G. Zühlsdorff, *ibid.*, **536**, 204 (1938).

(6) M. Müller, *Z. physiol. Chem.*, **233**, 223 (1935).

(7) *Cf.* W. P. Campbell, M. D. Soffer and T. R. Steadman, THIS JOURNAL, **64**, 425 (1942).

(8) F. Feist, *Ann.*, **496**, 99 (1932).

(9) D. E. Read and C. B. Purves, THIS JOURNAL, **74**, 116 (1952).

(10) G. M. Badger, W. Carruthers, J. W. Cook and R. Schoental, *J. Chem. Soc.*, 169 (1949).

the reason for the differing resistance of the methyl groups to oxidation in the two series. Alder and Krüger[11] have recently reported the synthesis of III (m.p. of the methyl ester 80°) from β-methylmuconic acid by addition of maleic anhydride and subsequent dehydrogenation.

The 9-methyl-*s*-octahydrophenanthrene (VI)[12] used in this study was prepared from 9-carboxyphenanthrene (VIIa). The methyl ester VIIb was reduced catalytically to the corresponding *s*-octahydro compound VIII and this on treatment with lithium aluminum hydride yielded the previously unreported 9-hydroxymethyl-*s*-octahydrophenanthrene (IX). With dry hydrogen chloride the latter was converted to the corresponding chloromethyl compound (X) of Badger, *et al.*,[13] and thence by reductive dehalogenation to VI.

The assignment of formula II to the methyltetracarboxybenzene (m.p. of the methyl ester 123°) of Inhoffen[3] and others[4-6] shows that the anthrasteroid rearrangement[2] takes place during the oxidation of steroids which contain two double bonds in ring B.

Experimental[14]

Oxidation of Dehydroergosteryl Acetate (Ib).—The oxidation was carried out essentially as reported by Inhoffen[3] for the free alcohol. Concentrated (70%) aqueous nitric acid (45 ml.) was added to 2.0 g. of dehydroergosteryl acetate (Ib) and the mixture was warmed. After the initial vigorous reaction had subsided, the red solution was refluxed gently for 16 hours. The solution became pale yellow and 35 ml. was removed by distillation. The solution was refrigerated at 0° for two hours to yield 175 mg. of slightly yellow crude acid. This was esterified with an excess of diazomethane. After crystallization from about 1 ml. of methanol the product weighed 115 mg. and melted at 121–124°. Recrystallization from methanol–water gave colorless needles, m.p. 122–124°. Inhoffen[3] gives m.p. 123–124°.

Oxidation of 9-Methyl-*s*-octahydroanthracene (V).—Oxidation of 0.77 g. of 9-methyl-*s*-octahydroanthracene (V) with 16 ml. of aqueous 70% nitric acid for 16 hours under reflux yielded 0.12 g. of crude acid after 8 ml. of the pale yellow solution was removed by distillation. The tetramethyl ester, prepared with excess diazomethane, melted at 123–125° after several recrystallizations from methanol and methanol–water. A mixture with the ester from dehydroergosteryl acetate melted at 122–124°. The infrared spectra of the two preparations were identical.

Anal. Calcd. for $C_{15}H_{16}O_8$: C, 55.55; H, 4.97. Found: C, 55.53; H, 4.97.

Oxidation of 9-Methyl-*s*-octahydrophenanthrene (VI).—Oxidation of 2.0 g. of 9-methyl-*s*-octahydrophenanthrene using 35 ml. of aqueous 70% nitric acid for 16 hours under reflux yielded 0.45 g. of crude acid. The corresponding ester prepared with diazomethane melted at 149–150°. The infrared spectrum of this ester (taken as a Nujol mull) was identical with that of pentacarbomethoxybenzene. The standard spectrum was obtained from Samuel P. Sadtler and Sons, Inc. The literature[9] records a melting point of 149–150° for pentacarbomethoxybenzene.

Anal. Calcd. for $C_{16}H_{16}O_{10}$: C, 52.17; H, 4.37. Found C, 52.33; H, 4.43.

A similar oxidation using 1.0 g. of the compound to 40 ml. of nitric acid yielded the same compound. It was also obtained when an insufficient amount of nitric acid was used followed by reoxidation.

9-Carbomethoxyphenanthrene (VIIb).—9-Phenanthroic acid (VIIa) (50 g.) was placed in a 3-liter flask with 2 liters of methanol and gaseous hydrogen chloride was passed through for 4.3 hours. The acid dissolved slowly in the methanol which warmed spontaneously. The ester crystallized during the latter half of the reaction period. The reaction mixture was refrigerated overnight at −20° to yield needles, m.p. 114–116°. The product was recrystallized from acetone–methanol to yield 33 g. (62%) of needles, m.p. 114–116° (lit. m.p. from 114.5–116°[15]).

9-Carbomethoxy-*s*-octahydrophenanthrene (VIII).—A solution of 20.0 g. of 9-carbomethoxyphenanthrene was hydrogenated in 400 ml. of glacial acetic acid with 4.0 g. of Adams platinum catalyst. After the absorption of about 109% of the theoretical amount of hydrogen (8 hours), the catalyst was removed by filtration, and the solvent was removed completely in a vacuum. The oily residue was crystallized from 150 ml. of methanol by refrigerating it several hours at −20°. The resulting block-like crystals weighed 15.9 g. (77%), m.p. 48–49° (lit. m.p. 45°[16]).

9-Hydroxymethyl-*s*-octahydrophenanthrene (IX).—To a solution of 7.4 g. of 9-carbomethoxy-*s*-octahydrophenanthrene in 40 ml. of dry ether was added slowly with stirring 60 ml. of *ca.* 1.2 *M* ethereal lithium aluminum hydride. The initial reaction caused the solution to reflux and the addition was made slowly (*ca.* 10 min.). The mixture, containing a voluminous precipitate, was stirred for 30 minutes after the addition was complete. Eighty ml. of 6 *N* aqueous HCl was added cautiously while cooling the mixture in an ice-bath. The resulting two clear layers were separated, and the organic layer was extracted with water until it was neutral. The ethereal solution was dried over sodium sulfate, evaporated nearly to dryness on the steam-bath and then 25 ml. of light petroleum ether was added. Precipitation began almost immediately and after a little while the mixture was refrigerated overnight at −20°. The product consisted of long, colorless, silken needles, m.p. 74–75°, weighing 6.0 g. (92%).

Anal. Calcd. for $C_{15}H_{20}O$: C, 83.28; H, 9.31. Found: C, 83.37; H, 9.17.

Another crystalline modification was also obtained. It formed short prisms, m.p. 82–83°.

Anal. Calcd. for $C_{15}H_{20}O$: C, 83.28; H, 9.31. Found: C, 83.60; H, 9.57.

Both modifications have been obtained either from light petroleum ether containing a little ether or from methanol–water. When the lower melting crystals were melted and allowed to cool from about 79°, they remelted at 80–81°. The compound had an absorption maximum in the ultraviolet at 272 mμ (ε 379) and a marked inflection at 280 mμ (ε 290).

9-Chloromethyl-*s*-octahydrophenanthrene (X).—Dry hydrogen chloride was passed into a solution of 5.5 g. of 9-hydroxymethyl-*s*-octahydrophenanthrene in 80 ml. of dry benzene. The mixture was vigorously stirred in the presence of 12.0 g. of 12-mesh anhydrous calcium chloride. After four hours the solution was filtered and evaporated to dryness in a vacuum. Crystallization from 10 ml. of light petroleum ether at −20° yielded a solid mass of colorless crystals from which the mother liquor was decanted. The product weighed 5.6 g. (94%) and melted at 56.5–57.5°. Badger, *et al.*,[13] give m.p. 56°. Its ultraviolet absorption spectrum was characterized by maxima at 272 mμ (ε 560) and at 278 mμ (ε 570) and an inflection at 284–286 mμ (ε 510).

9-Methyl-*s*-octahydrophenanthrene (VI).—A solution of 5.6 g. of 9-chloromethyl-*s*-octahydrophenanthrene in 100 ml. of glacial acetic acid was hydrogenated using 0.414 g. of Adams platinum catalyst. One molar equivalent of hydrogen was absorbed in less than 60 minutes. The catalyst was removed by filtration and the acetic acid by distillation

(11) K. Alder and B. Krüger, *Ber.*, **86**, 985 (1953).

(12) Badger, *et al.*,[13] have prepared this compound from *s*-octahydrophenanthrene by chloromethylation and reductive dehalogenation. This method, however, gave poor yields and the product was difficult to purify.

(13) G. M. Badger, W. Carruthers and J. W. Cook, *J. Chem. Soc.*, 2044 (1949).

(14) All melting points have been taken on an electrically heated block (Kofler) and are uncorrected. Ultraviolet spectra were determined in isoöctane on a Cary spectrophotometer. Infrared spectra were determined on a Perkin-Elmer double beam spectrophotometer by Mrs. Alma Hayden. Microanalyses are by the Analytical Service Laboratory of this Institute under the direction of Dr. William C. Alford.

(15) (a) E. Mosettig and J. van de Kamp, THIS JOURNAL, **52**, 3704 (1930); (b) C. W. Shoppee, *J. Chem. Soc.*, 37 (1933); (c) D. S. Tarbell and Y. Sato, THIS JOURNAL, **68**, 1091 (1946).

(16) J. van de Kamp and E. Mosettig, *ibid.*, **57**, 1107 (1935).

in vacuum. The residue was dissolved in ether, extracted with a sodium carbonate solution, washed with water, dried over sodium sulfate and evaporated to dryness in a vacuum. The oily residue was distilled. The product was collected at 131–132° at 0.8–0.9 mm. and weighed 3.4 g. (71%). It was a clear, colorless oil, λ_{max} 271, 277 and 281 mμ (ε 399, 331 and 355), λinfl. 263 mμ (ε 252), $n^{20}D$ 1.5663, m.p. 17° (as determined by the plateau in the warming curve). Badger, *et al.*,[13] give only b.p. 106–110° (air-bath temperature) (0.3 mm.).

Anal. Calcd. for $C_{15}H_{20}$: C, 89.93; H, 10.06. Found: C, 90.05; H, 10.25.

9-Methyl-*s*-octahydroanthracene (V).—This was prepared essentially according to the procedure of Badger, *et al.*,[10] from *s*-octahydroanthracene. The latter was prepared by the reduction[17] of purified anthracene in absolute ethanol using Raney nickel at 900 p.s.i. and 145°. The intermediate **9-chloromethyl-*s*-octahydroanthracene** melted at 90–91°, λ_{max} 292 mμ (ε 1780), λinfl. 286–288 mμ (ε 1760). Badger, *et al.*,[10] gave m.p. 91–92°. It was dehalogenated by hydrogenation in ethanol at room temperature and atmospheric pressure using 10% by weight of 5% palladium-on-charcoal. One molecular equivalent of hydrogen was absorbed in 30 minutes. The product consisted of lustrous flakes, m.p. 50.5–51.5°, λ_{max} 274, 279 and 283 mμ (ε 664, 548 and 720), λ infl. 265 mμ (ε 350). Badger, *et al.*,[10] give m.p. 52°.

(17) (a) H. I. Waterman, J. J. Leendertse and A. C. Cranedonk. *Rec. trav. chim.*, **58**, 83 (1939); (b) G. Schroeter, *Ber.*, **57**, 2003 (1924).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

A New Synthesis of 2-Phosphoryl-D-glyceric Acid

BY CLINTON E. BALLOU AND HERMANN O. L. FISCHER

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A definitive synthesis of 2-phosphoryl-D-glyceric acid is described which yields the pure compound in quantity as the crystalline trisodium salt. Although the rotation of this synthetic material is in disagreement with that of previously reported preparations of 2-phosphoryl-D-glyceric acid, chemical and enzymatic studies have given conclusive proof of the identity and purity of this preparation.

2-Phosphoryl-D-glyceric acid (2PGA), the glycolytic intermediate between 3-phosphoryl-D-glyceric acid (3PGA) and phosphoryl enolpyruvic acid (PEPA), was first isolated by Meyerhof and Kiessling from yeast,[1] and was characterized as a crystalline barium salt whose analysis indicated the empirical formula $C_3H_5O_7PBa \cdot 1^1/_2\ H_2O$. When dissolved in 1 *N* hydrochloric acid, the substance showed $[\alpha]D$ +24.3°, the concentration being calculated on the basis of the free acid. Meyerhof and Kiessling[1] also described the preparation of unnatural 2-phosphoryl-L-glyceric acid from synthetic 2-phosphoryl-DL-glyceric acid[2] by treatment with a muscle extract which metabolized the natural substance. The L-isomer remaining was isolated as a crystalline barium salt, $C_3H_5O_7PBa \cdot 3H_2O$, and under the same conditions described above showed $[\alpha]D$ −23.6°.

A second synthesis of 2-phosphoryl-D-glyceric acid has been described by Neuberg, who treated methyl D-glycerate with ethyl metaphosphate.[3] From the mixture of phosphorylated compounds he isolated, by a prolonged fractionation of metal salts, a small yield of 2-phosphoryl-D-glyceric acid as the barium salt (no analysis), which was converted to a silver salt, $C_3H_4O_7PAg_3$. The barium salt in 1 *N* acid showed $[\alpha]D$ +23.2°.

We are reporting in this paper the first definitive synthesis of 2-phosphoryl-D-glyceric acid, which affords the natural isomer in quantity in an optically pure form as the easily crystallized trisodium salt (pentahydrate). Since the rotation which we have observed for our preparation, $[\alpha]D$ +13° (1 *N* hydrochloric acid), is in disagreement with that reported by previous workers, we will outline the synthesis and characterization of the compound in detail.

Our preparation of 2-phosphoryl-D-glyceric acid was carried out by phosphorylation of methyl 3-*O*-benzyl D-glycerate. This intermediate was prepared from D-galactose by (1) acetonation to 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose; (2) benzylation of this compound to give 1,2:3,4-di-*O*-isopropylidene-6-*O*-benzyl D-galactopyranose; (3) conversion of the benzylated compound to methyl 6-*O*-benzyl α-D-galactopyranoside with methanolic hydrogen chloride; (4) cleavage of this compound with periodate, followed by oxidation of the dialdehyde to the dicarboxylic acid, which was hydrolyzed to glyoxylic acid and 3-*O*-benzyl D-glyceric acid; (5) isolation of 3-*O*-benzyl D-glyceric acid as the crystalline calcium salt; and (6) conversion of the calcium salt to methyl 3-*O*-benzyl D-glycerate with diazomethane. The optical purity of the 3-*O*-benzyl D-glyceric acid and its methyl ester were established by debenzylation to give D-glyceric acid with the recorded specific rotation.

Phosphorylation of methyl 3-*O*-benzyl D-glycerate[4] was carried out with diphenylphosphoryl chloride. The blocking groups were removed from the methyl 3-*O*-benzyl-2-diphenylphosphoryl D-glycerate by treatment first with hydrogen and palladium, followed by hydrogen and platinum. The resulting methyl 2-phosphoryl-D-glycerate was saponified, and the acid then crystallized as the trisodium salt from water by the addition of methanol. The over-all yield from D-galactose was about 20%.

Characterization of this synthetic material has been effected as follows: Elemental analyses on the hydrate and on the anhydrous material are in excellent agreement with the calculated values. Dephosphorylation with acid according to Kiessling and Schuster,[4a] or with intestinal phosphatase gave D-glyceric acid with the recorded rotation, thus es-

(1) O. Meyerhof and W. Kiessling, *Biochem. Z.*, **276**, 239 (1935).
(2) W. Kiessling, *Ber.*, **68**, 243 (1935).
(3) C. Neuberg, *Arch. Biochem.*, **3**, 105 (1943).
(4) We have also used this substance as a starting material for a definitive synthesis of 3-phosphoryl-D-glyceric acid.
(4a) W. Kiessling and P. Schuster, *Ber.*, **71**, 123 (1938).