Anhydrous ammonia was passed for 1 hr. at 0° into 25 ml. of methanol containing 3.38 g. of V. The solution was kept in a refrigerator for 21 hr. and was then evaporated under reduced pressure to a sirup. The sirup was dissolved in 60 ml. of water and washed with chloroform. The water extract was concentrated to dryness. The solid material was twice triturated with methanol. The methanol solution was decolorized and allowed to stand overnight at room temperature. Two crops of crystalline 2-acetamido-9-lactosyladenine (VII) were obtained; combined yield 400 mg., m.p. 263–267°; absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 224, 270 mμ; ν_{max}^{KBr} 3400, 3320 cm.$^{-1}$ (OH, NH), 1650 cm.$^{-1}$ (amide carbonyl or C=C), 1620 cm.$^{-1}$ (NH_2 and H_2N—C=N), 1120, 1075, 1030, 990 cm.$^{-1}$ (C–O–C and C–O–H).

Anal. Calcd. for $C_{19}H_{28}N_6O_{11}$: C, 44.17; H, 5.48; N, 16.27. Found: C, 44.26; H, 5.50; N, 16.29.

2,6-Diamino-9-β-lactosylpurine (VIII).—Complete deacetylation of 2,6-diacetamido-9-(hepta-*O*-acetyl-β-lactosyl)-purine (V) was effected by boiling with an excess of sodium methoxide in methanol.[12] A small pellet of freshly cut sodium was added to a solution of 12 g. of V in 40 ml. of dry methanol containing 60 ml. of 0.1 *N* sodium methoxide. The mixture was refluxed for 2 hr., cooled and neutralized with acetic acid. The solution was evaporated to dryness under reduced pressure, the residue was dissolved in 100 ml. of water and washed with chloroform. After concentration to 20 ml., 60 ml. of a 10% methanolic solution of picric acid was added. The crystalline picrate complex formed immediately; yield 8.0 g., dec. 270–275°.

The yellow crystalline picrate (8.0 g.), was suspended in 200 ml. of warm water and stirred with Dowex-1 (carbonate form) anion exchange resin[13] until the solution became colorless. The solution was filtered and concentrated to dryness to produce crude crystalline 2,6-diamino-9-β-lactosylpurine (VIII); yield 2.09 g. (45%). The pure material was obtained upon recrystallization from water; m.p. 283–285°, $[\alpha]^{22}D$ −5.5° (*c* 0.5, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 256, 280 mμ; ν_{max}^{KBr} 3440, 3380, 2900 cm.$^{-1}$ (OH, NH), 1640, 1615 cm.$^{-1}$ (NH_2 and purine ring), 1088, 1065, 1040, 1005 cm.$^{-1}$ (C–O–C and C–OH); X-ray powder diffraction data[11]: 11.6m, 7.34vw, 6.03m, 5.75vs(1), 5.27m(2), 5.03w, 4.77vw, 4.51w, 4.23vs(1), 3.96vw, 3.78m(3), 3.61vs(1).

Anal. Calcd. for $C_{17}H_{26}N_6O_{10}$: C, 43.03; H, 5.53; N, 17.72. Found: C, 42.91; H, 5.48; N, 17.73.

(12) B. R. Baker and Kathleen Hewson, *J. Org. Chem.*, **22**, 959 (1957).

(13) A product of the Dow Chemical Co., Midland, Mich.

COLUMBUS 10, OHIO

COMMUNICATIONS TO THE EDITOR

THE CONFIGURATION OF B-NORSTEROID DERIVATIVES

Sir:

In 1956,[1] this laboratory reported results in the B-norsteroid series which when compared to those in normal steroids indicated that the stable configuration of the A/B ring juncture in the former series was *trans*. Subsequently, on the basis of rotatory dispersion measurements, Djerassi, Marshall and Nakano[2] suggested a *cis* arrangement and recently on the basis of chemical transformations Goto and Fieser[3] arrived at a similar conclusion. We wish to report the results of our continuing study which are in agreement with the latter conclusion and which define certain other stereochemical aspects of this system.

Hydrogenation of B-norcholesteryl acetate (I) in acetic acid over platinum and then saponification yielded 70% of 3β-hydroxy-B-norcoprostane (II, m.p. 77°, $[\alpha]D$ +16°, formerly called B-norcholestanol) and 15% of 3β-hydroxy-B-norcholestane (III, m.p. 132°, $[\alpha]D$ +8°). Oxidation of II and III yielded the corresponding 3-keto derivatives (m.p. 76°, $[\alpha]D$ +16°; m.p. 96°, $[\alpha]D$ +35°), the rotatory dispersion curves of which were practically identical with coprostanone and cholestanone, respectively.[4] Reduction of B-norcoprostane-3-one with LAH gave rise to 25% of II and 75% of the 3α epimer IV (m.p. 96°, $[\alpha]D$ +16°); previously we had reported a predominance of II. Thus, on the basis of the dispersion curves and the stereochemistry of the hydride reduction, II is A/B *cis* and III is A/B *trans*. This hydrogenation result differs greatly from that of cholesteryl acetate which yields mostly the A/B *trans* material.

The direct chemical method employed by Goto and Fieser to establish a *cis* configuration in B-norcoprostane-3,6-dione (V) was the conversion with $NaBH_4$ of the dione V to a 3,6-diol (VI) assumed to be 3β,6α, which, in turn, was transformed into a 3α,6α-oxide (VII). The diketone V has only been related to the B-nor series obtained by hydrogenation of I on the basis of Wolff-Kishner reduction to the same hydrocarbon (VIII) obtained from B-norcoprostanone. Since it is known that such a reduction of a carbonyl group can affect adjacent asymmetric centers,[5] a stereospecific conversion method must be used before the oxide data can be employed for the configurational determination of II. LAH reduction of 3,6-dione V yielded four diols, epimeric only at the hydroxyl functions, two of these diols (VI, m.p. 141° and IX, m.p. 171°) having been reported by Goto and Fieser. The diol IX was converted to a ditosylate (m.p. 121°) which upon reduction with LAH yielded B-norcoprostane VIII, thus specifically relating II and V.

Finally, Goto and Fieser postulated conformation XI as the preferred conformation of compounds in this series on the basis of our earlier LAH reduction results. In addition, they also preferred XI on energetic grounds and postulated that oxide VII was derived directly from conformation XI, ring A remaining as a chair. We prefer conformation X, the usual steroidal conformation,

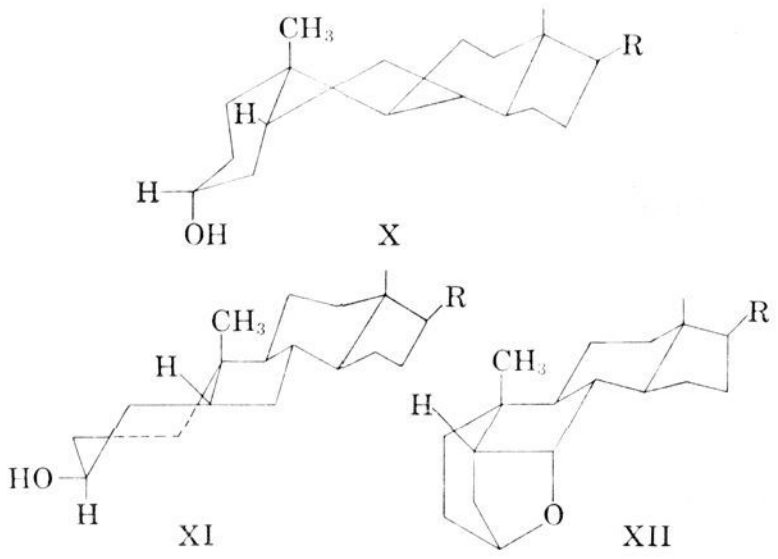
(1) W. G. Dauben and G. J. Fonken, THIS JOURNAL, **78**, 4386 (1956).

(2) C. Djerassi, D. Marshall and T. Nakano, *ibid.*, **80**, 4856 (1958).

(3) T. Goto and L. F. Fieser, *ibid.*, **81**, 2276 (1959).

(4) Kindly performed by Professor C. Djerassi.

(5) C. Djerassi, R. Riniker and B. Riniker, THIS JOURNAL, **78**, 6362 (1956).



for the reasons given. First, revision of our hydride results clearly indicates that the 3α configuration is equatorial. Second, from a study of models, it is clear that an oxide derived from conformation XI is impossible and that ring A must be in a boat conformation (XII) in the oxide.[6] Thirdly, using the "octant rule,"[7] it is predicted[8] that the rotatory dispersion curve of conformation X should be negative and of conformation XI should be positive; the dispersion curve of B-norcoprostanone is negative.

Acknowledgment.—Investigation supported, in part, by U. S. Public Health Grant CY-4284.

(6) Prof. L. F. Fieser has informed us that he is in agreement with this conclusion as regards the conformation of the oxide.

(7) W. Moffitt, A. Mascowitz, R. B. Woodward, W. Klyne and C. Djerassi, to be published.

(8) R. B. Woodward, private communication.

(9) General Electric Co. Fellow in Chemistry, 1958–1959.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

WILLIAM G. DAUBEN
GEORGE A. BOSWELL, JR.[9]
GILBERT H. BEREZIN

RECEIVED OCTOBER 2, 1959

ENZYMATIC CONVERSION OF GLUCOSE-6-PHOSPHATE (G6P) TO A NEW HEPTULOSE PHOSPHATE BY RAT LIVER[1]

Sir:

Rat liver preparations and hemolysate have been reported to convert G6P to sedoheptulose-7-phosphate on the basis of color reactions[2] and paper chromatography[3] of the free heptulose. We have now found another product of G6P metabolism, a new heptulose phosphate which can be readily distinguished on paper from sedoheptulose phosphate (Fig. 1). In addition, the migration of the anhydride of the new heptulose was more rapid than sedoheptulosan in acetone, butanol, water (7:2:1). However, the free sugars cannot be separated on paper, which accounts for the delay in observing the new heptulose. Evidence for its structure will be reported in a later communication.

(1) Aided by research grants from the American Cancer Society, Inc., New York (P-106, P-107), and the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. (C-3213).

(2) Z. Dische, *Ann. N. Y. Acad. Sci.*, **75**, 129 (1958).

(3) A. Bonsignore, S. Pontremoli, G. Fornaini and E. Grazi, *Boll. Soc. ital. biol. sper.*, **33**, 555, 558 (1957).

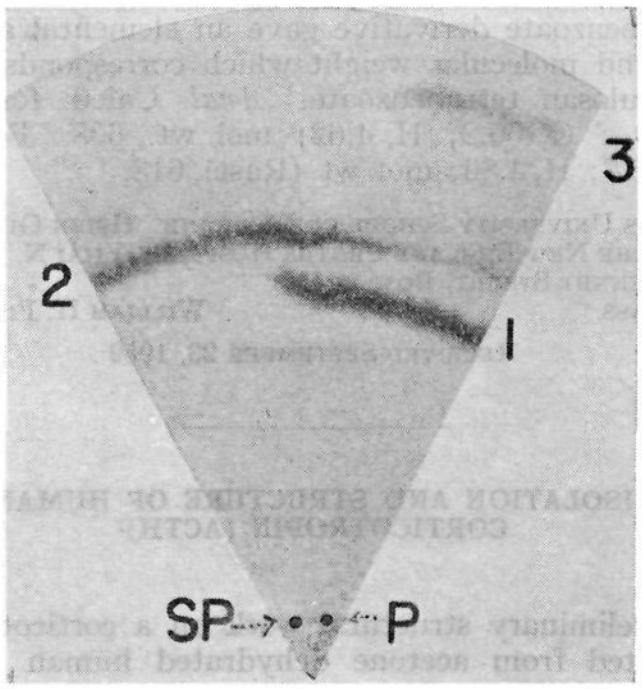


Fig. 1.—A sector of a circular paper (Whatman no. 3 MM) chromatogram (radius 14 cm.) showing the difference in mobility between S7P and the heptulose phosphate from the G6P reaction mixture. Solvent: ethyl acetate, pyridine, acetic acid, water (5:3:1:1) as developing solvent and ethyl acetate, pyridine, water (11:40:6) for saturation of the chromatographic chamber[7]; time, 10 hours. Multiple development was carried out[8]: spraying reagent, 0.5% orcinol in 15% TCA solution in water-satd. butanol, specific for ketoheptoses only.[9] SP = Sedoheptulose-7-phosphate (a gift from Dr. B. L. Horecker) travelled 7.2 cm. to position 2 and the product (P) of the deproteinized and concentrated reaction mixture of G6P, 6.1 cm. to position 1. Position 3 is the location of free heptuloses.

In a typical reaction, 0.2 ml. of 0.1 *M* G6P solution plus 0.5 ml. of Tris buffer, *p*H 7.2, was incubated with 0.3 ml. of a supernatant solution obtained by centrifuging a 10% liver homogenate for 30 minutes at 18,000 × *g*. After 4 hours at 37° in the presence of toluene the mixture was deproteinized either by adding 1 ml. of 95% ethanol or by heating at 100° for 2 minutes and the supernatant was analyzed for heptulose by the Dische orcinol reaction.[4]

Additional evidence for the existence of a new ketoheptose is provided by the isolation from a large scale digest[5] of heptulosan anhydride which failed to crystallize like sedoheptulosan but gave a crystalline tetrabenzoate (20 mg.) (spheroids) whose melting point (75–76°) and optical rotation (no rotation at 2.9% in $CHCl_3$) differed from sedoheptulosan tetrabenzoate[6] (hexagonal plates) melting point (164–165°) and $[\alpha]^{25}D$ −188°. The

(4) Z. Dische, *J. Biol. Chem.*, **204**, 983 (1953).

(5) The protein free filtrate from 250 ml. of digest (substrate; 1 g. G6P) was chromatographed on a cellulose column and developed with methyl Cellosolve, methyl ethyl ketone, ammonia water (7:2:3). Heptose-phosphate rich fractions were pooled and their aqueous solutions hydrolyzed with potato phosphatase. In each case the free heptose was further purified by cellulose column chromatography (acetone, butanol, water, 7:2:1) until a single sugar (paper chromatography) was the product. By treatment with hot mineral acid the heptose was converted to the anhydride.

(6) V. N. Nigam, Hsien-Gieh Sie and W. H. Fishman, *J. Biol. Chem.*, **234**, 1955 (1959).

(7) F. G. Fischer and Helmut Dörfel, *Z. physiol. Chem.* **301**, 224 (1955).

(8) K. V. Giri and V. N. Nigam, *J. Ind. Inst. Sci.*, **36**, 49 (1954).

(9) R. Kleostrand and A. Nordal, *Acta Chem. Scand.*, **4**, 1320 (1950).