benzaldehyde. In each case, the diazoalkane was formed, although the conversions were not as good as that observed in the fluorenone case.[8,9]

In an attempt to develop this finding into a more useful synthetic technique, some preliminary experiments were carried out in which hydroxylamine-O-sulfonic acid (IV), a crystalline solid,[10] was substituted for chloramine. Fluorenone oxime was found to react with IV in aqueous base to give diazofluorene in *ca.* 60% yield. Similarly, benzophenone oxime gave diphenyldiazomethane (30%), while oximes of acetophenone and benzaldehyde gave small amounts of diazoalkanes.[9]

These results clearly indicate that a carbonyl group is not essential for the >C=NOH → >C=N_2 conversion, and therefore support a mechanism of type (1) for the Forster diazoketone synthesis.

Acknowledgment.—This work was supported in part by a grant from the National Science Foundation.

(8) It has been reported recently by L. A. Carpino, C. A. Giza and B. A. Carpino, THIS JOURNAL, **81**, 955 (1959), that "attempts to apply the Forster reaction to the synthesis of simple diazo compounds such as diazofluorene were unsuccessful." This failure to observe the desired reactions may possibly be a consequence of an unfortunate choice of experimental conditions.

(9) It is interesting to note that as by-products, benzhydrol was found to accompany the diphenyldiazomethane, and benzonitrile was formed along with phenyldiazomethane.

(10) F. Sommer, O. F. Schulz and M. Nassau, *Z. anorg. u. allgem. Chem.*, **147**, 142 (1925); G. Gever and K. Hayes, *J. Org. Chem.*, **14**, 813 (1949).

(11) Fellow of the Alfred P. Sloan Foundation.

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RECEIVED JUNE 4, 1959

SYNTHESIS OF THE TWO ANOMERIC 9-(2-DEOXY-D-RIBOFURANOSYL)-ADENIVEU*

Sir:

The several methods[1,2,3] for the synthesis of 2′-deoxynucleosides all involve rather complicated alterations of the functional groups of pentofuranosyl moieties already bound to purines or pyrimidines. The relative accessibility of 2-deoxy-D-ribose[4] makes a direct approach to the synthesis of 2′-deoxynucleosides from this sugar potentially more attractive. This Communication reports such an approach, the immediate objective being the synthesis of natural 2′-deoxyadenosine [9-(2-deoxy-β-D-ribofuranosyl)-adenine] (I) and its unnatural anomer (II).

2-Deoxy-D-ribose was converted to its amorphous diisobutyl mercaptal[5] and then mono-*p*-nitrobenzoylated to give crystalline 2-deoxy-5-*O*-*p*-nitrobenzoyl-D-ribose diisobutyl mercaptal (III) in 44% yield: m.p.[6] 74–75°, $[\alpha]^{20}D$ −10.2° (*c* 0.81, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{31}NO_6S_2$: C, 53.91; H, 7.01; N, 3.14. Found: C, 53.68; H, 6.91; N, 3.33. Demercaptalation of III yielded an amorphous product which then was fully acylated with *p*-nitrobenzoyl chloride in pyridine solution to give the two crystalline, anomeric 2-deoxy-1,3,5-tri-*O*-*p*-nitrobenzoyl-D-ribofuranoses (α-IV and β-IV) in a total yield of 82% based on III. One isomer, m.p. 164–165°, $[\alpha]^{20}D$ +69.9° ($CHCl_3$, *c* 0.66), is assigned structure α-IV. *Anal.* Calcd. for $C_{26}H_{19}N_3O_{13}$: C, 53.71; H, 3.29; N, 7.23. Found: C, 53.99; H, 3.56; N, 7.21. The second isomer, m.p. 172–173°, $[\alpha]^{20}D$ +17° ($CHCl_3$, *c* 0.36), is assigned structure β-IV. Found: C, 53.98; H, 3.57; N, 7.15.

Either α-IV or β-IV or a mixture of the two was found to serve equally well in the following steps. The ester, IV, was dissolved in methylene chloride and treated with a slight excess of hydrogen chloride, the precipitated *p*-nitrobenzoic acid (87%) filtered off and the solvent removed *in vacuo*. To the residue was added a solution of chloromercuri-6-benzamidopurine in anhydrous dimethyl sulfoxide. After two hours at room temperature, the product was precipitated with water and, after drying, treated with methanolic barium methoxide. The deacylated material was chromatographed on powdered cellulose using isopropyl ether–ethanol–water (16:4.5:1 v./v.). Adenine, I and II appeared in this order. Solvent was evaporated from I, the crystalline residue leached with methylene chloride and the remainder crystallized from water to give pure material which was dried *in vacuo* at 110° for 5 hr. (8.3% yield). After recrystallization from water it showed m.p. 191–194°, $[\alpha]^{25}_{589}$ − 25°; $[\alpha]^{25}_{450}$ −59°; $[\alpha]^{25}_{400}$ −72°; $[\alpha]^{25}_{360}$ −104°; $[\alpha]^{25}_{340}$ −127°; $[\alpha]^{25}_{330}$ −137° (H_2O, *c* 0.47). *Anal.* Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 47.73; H, 5.28; N, 27.36 (sample dried 5 hr. at 100° *in vacuo*).

Authentic 9-(2-deoxy-β-D-ribofuranosyl)-adenine melts at 191–192° and does not depress the melting point of the synthetic product. A repurified commercial sample of I showed: $[\alpha]^{25}_{589}$ −26°; $[\alpha]^{25}_{400}$ − 71°; $[\alpha]^{25}_{360}$ −103°; $[\alpha]^{25}_{340}$ −128°; $[\alpha]^{25}_{330}$ −150°; $[\alpha]^{25}_{320}$ −173°; $[\alpha]^{25}_{310}$ −206° (H_2O, *c* 0.49). Both natural and synthetic I showed absorption peaks at 260 mμ, A_M for natural I being 15,900 and A_M for synthetic I being 16,600.

The infrared spectra of both were never found to be completely identical using the KBr-plate technique. However, we have observed that 2′-deoxyadenosine crystallizes with varying amounts of water and possibly in dimorphic forms, both natural and synthetic specimens even after rigorous drying often showing a m.p. of *ca.* 160–170° before finally melting at 191–192°.

The anomeric nucleoside (II), obtained in 18% yield, was recrystallized successively from ethanol–pentane, ethanol and methanol, m.p. 209–211°, $[\alpha]^{25}_{589}$ +71°; $[\alpha]^{25}_{450}$ +132°; $[\alpha]^{25}_{400}$ +173°;

* This Communication to the Editor was received prior to that of M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung (THIS JOURNAL, **81**, 4112 (1959)) and was to have been published simultaneously; unfortunately the manuscript was filed pending acceptance of the later Communication, and then overlooked and not put in process of publication until after the other had appeared.—The Editors.

(1) D. M. Brown, D. B. Pariher, C. B. Reese and A. Todd, *J. Chem. Soc.*, 3035 (1958).

(2) G. Shaw and R. N. Warrener, *ibid.*, 50 (1959).

(3) C. D. Anderson, L. Goodman and B. R. Baker, THIS JOURNAL, **80**, 6453 (1958).

(4) H. W. Diehl and H. G. Fletcher, Jr., *Arch. Biochem. Biophys.*, **78**, 386 (1958).

(5) H. Zinner, H. Nimz and H. Venner, *Chem. Ber.*, **90**, 2696 (1957).

(6) Melting points are corrected.

$[\alpha]^{25}_{360}$ +220°; $[\alpha]^{25}_{340}$ +258° [H_2O, *c* 0.54). *Anal.* Calcd. for $C_{10}H_{13}N_5O_8$: C, 47.80; H, 5.22; N, 27.88. Found: C, 48.01; H, 5.49; N, 27.74. Like its anomer, the substance shows an absorption peak at 260 mμ characteristic of a 9-substituted adenine,[7] the molar absorbancy (A_M) being 15,900.

Hydrolysis of a sample with 1% aqueous acetic acid, and then paper chromatography in four different solvent systems, revealed the presence of adenine, 2-deoxy-D-ribose and unchanged nucleoside.

(7) J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 259 (1938).

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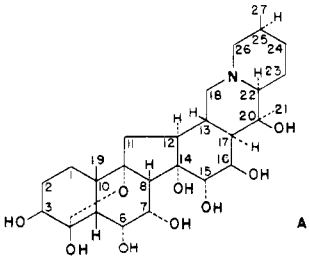
RECEIVED MAY 18, 1959

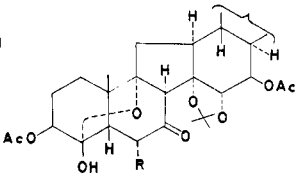
VERATRUM ALKALOIDS. XXXIV. THE CONFIGURATION OF PROTOVERINE[1]

Sir:

Protoverine[2-4] is the alkamine obtained by alkaline hydrolysis of the clinically useful[5] hypotensive ester alkaloids protoveratrine A[6] and protoveratrine B.[6] Evidence is advanced herewith for assignment of configuration at each of the seventeen asymmetric centers of protoverine which now can be represented completely by formula I.



I



II, R = OAc
III, H = H

The orientations at fourteen of the asymmetric carbon atoms of protoverine have been established by a single degradation. Treatment of 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (II)[4] in tetrahydrofuran with calcium in liquid ammonia[7] afforded the known[8] 7-dehydrogermine 14,15-acetonide 3,16-diacetate (III). The configurations at C_3, C_4, C_5, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{20}, C_{22}, and C_{25} are therefore the same as those at the corresponding asymmetric carbon atoms in germine.[8]

The steric hindrance to acetylation of the C_7-hydroxyl group of protoverine by the α-oriented 14,15-acetonide grouping[4] is explicable uniquely on the basis of a C_8-β-hydrogen (as in all other naturally occurring steroids), C_7-α-hydroxyl configuration. Support for assignment of α-orientation to the C_7-hydroxyl is presented: (a) sodium borohydride reduction of II proceeded stereoselectively to give protoverine 14,15-acetonide 6,16-diacetate, m.p. 229–230° dec.; $[\alpha]^{25}_D$ +4° (*c* 0.95, pyr.). The latter compound consumed one mole equivalent of sodium periodate and yielded an amorphous product showing infrared absorption at 3.65 and 5.62 μ characteristic of an aldehydo-γ-lactone resulting from cleavage of the Ring A glycol.[4] Upon acetylation, the 14,15-acetonide 6,16-diacetate gave the known protoverine 14,15-acetonide 3,6,16-triacetate.[4] The molecular model of the ketone (II) shows that the β- is much less hindered than the α-face for approach to the borohydride, which suggests that reaction would proceed to give an α-oriented hydroxyl.[9] (b) Acetylation of protoverine with acetic anhydride-pyridine, reagents known to acetylate the C_4-hemiketal hydroxyl in veracevine,[10] afforded protoverine 3,6,7,15,16-pentaacetate,[4] consistent with rapid acetylation of the α-hydroxyl group at C_7 and resultant hindrance to reaction of the C_4-hydroxyl group by the 7-α-acetoxy group, (as in germine[8]).

Formation of the 6,7-acetonide derivative[4] of isoprotoverine requires that the C_6 hydroxyl group be oriented *cis* to the C_7-hydroxyl; hence protoverine possesses the 6-α-hydroxygermine structure and configuration (I).[11,12]

(1) Part XXXIII in the series: S. M. Kupchan and T. Masamune, *Chemistry and Industry*, 632 (1959).

(2) W. Poethke, *Arch. Pharm.*, **275**, 357, 571 (1937).

(3) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **149**, 271 (1943).

(4) S. M. Kupchan, M. Neeman, C. I. Ayres, R. H. Hensler and S. Rajagopalan, *Chemistry and Industry*, 1626 (1958).

(5) O. Krayer in V. A. Drill, "Pharmacology in Medicine," McGraw-Hill Book Co., Inc., New York, N. Y., Second Edition, 1958, pp. 515–524.

(6) S. M. Kupchan and C. I. Ayres, THIS JOURNAL, **81**, 1009 (1959), and references therein.

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(8) S. M. Kupchan and C. R. Narayanan, THIS JOURNAL, **81**, 1913 (1959).

(9) *Cf.* W. G. Dauben, G. J. Fonken and D. S. Noyce, *ibid.*, **78**, 2579 (1956).

(10) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **75**, 5519 (1953).

(11) Satisfactory analytical and spectral data were obtained for the new compound reported herein.

(12) This investigation was supported by grants from The National Institutes of Health (H-2275(C3)) and the Wisconsin Alumni Research Foundation.

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RECEIVED JULY 16, 1959

A NEW ASSAY METHOD FOR AMINO ACID ACTIVATING ENZYMES[1]

Sir:

We wish to report a new technique for the estimation of amino acid activating enzymes.[2] It is extremely simple to carry out, rapid, sensitive and conservative of all materials. This method permits assay of a specific amino acid activating enzyme in the presence of all other amino acids and activating enzymes (plus other hydroxamate forming or adenosine triphosphate-pyrophosphate exchanging systems). The method also permits the detailed study of competition between two or more amino acids both of which are activated by a single enzyme. Particularly in these latter two respects,

(1) This Publication No. 968 of the Cancer Commission of Harvard University; the work was supported by United States Public Health Grant No. C-2387 and by United States Atomic Energy Commission contract AT(30-1)609.

(2) M. B. Hoagland, *Biochim. et Biophys. Acta*, **16**, 288 (1955).