protons in each solvent were represented by a single peak. The C-6 protons in each instance appeared as a triplet (J = 5 c.p.s.).[14]

Attempted Hydrolysis of VI (VIa).—The material was heated for about 4 hr. with 18% hydrochloric acid, cooled, made basic, and extracted with ether. Similarly, hydrolysis with a 20% sodium hydroxide solution was carried out. The cooled solution was extracted with ether. The two extracts were dried and concentrated. The residues were examined. Their n.m.r. spectra were unchanged. However, when hydrolysis was carried out in 20% sodium hydroxide for 18–20 hr., a sample of the solution submitted for n.m.r. showed complete removal of the methyl protons.

Acknowledgment.—The authors are indebted to Dr. John Tadanier of the Organic Research Department of this laboratory and to Professor Peter Beak of the University of Illinois at Urbana for the discussions of the n.m.r. data while trying to develop the structure of VI (VIa).

(14) The spectrum of VI (VIa) in dimethyl sulfoxide to which a few drops of concentrated hydrochloric acid were added showed the N-methyl protons as a doublet, 2.83 and 2.92 (J = 5 c.p.s.), and the C-6 protons were seen as an unsharp group with separate peaks (J = 2 c.p.s.) suggestive of two overlapping triplets probably caused by protonation of the heterocyclic nitrogen. The spectrum in concentrated hydrochloric acid also showed a doublet, 2.89 and 2.97 (J = 5 c.p.s.), for the N-methyl protons and split peaks, 3.45 and 3.49 (J = 2 c.p.s.), amid an unsharp group for the C-6 proton signal. These two spectra only establish that the protonated form of VI (VIa) exists as a resonance stabilized hybrid.

Microbial Hydroxylation of 5,6-Dihydrosolasodine

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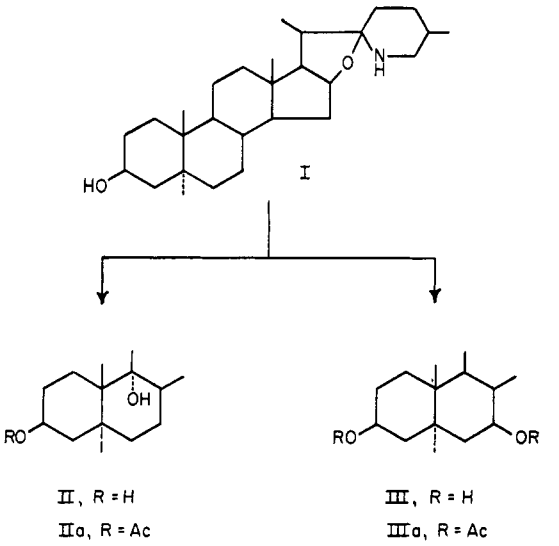
The hydroxylation of diosgenin, the well-known steroidal sapogenin,[2] with the fungus, *Helicostylum piriforme* (ATCC, 8992), leads to the formation of 7β,11α-dihydroxy- (10–15% yield) and 11α-hydroxy-7-oxodiosgenin (5–10% yield).[3] However, when the same fungus was incubated with the 5,6-dihydro derivative of diosgenin, *i.e.*, tigogenin, no detectable amount of hydroxylation was observed.[3]

In view of this somewhat surprising effect of the saturated 5,6-dihydro steroid in suppressing hydroxylation, it became of some interest to study the behavior of 5,6-dihydrosolasodine[4] (solasodan-3β-ol) since its precursor solasodine has previously been shown to hydroxylate readily to form 9α- (*ca.* 35%), 11α- (*ca.* 1%), and 7β-hydroxysolasodine (*ca.* 1%).[5]

The hydroxylation of 5,6-dihydrosolasodine (I), contrary to our expectation, was not altered to any appreciable degree in comparison with solasodine; the corresponding 9α-hydroxy- (II) and 7β-hydroxy-5,6-dihydrosolasodine (III) were obtained. The yields were roughly comparable with that obtained in the hydroxylation of solasodine. The diol II formed the expected O,N-diacetylhydroxy derivative, IIa, and III, the O,O,N-triacetate IIIa, upon acetylation.



The identity of diol II was determined by comparison with a sample of 9α-hydroxy-5,6-dihydrosolasodine prepared by the catalytic reduction of 9α-hydroxysolasodine.[5] The location of the hydroxyl function in the latter has been authenticated. The structure of the second diol III was likewise ascertained by comparison with a specimen of 7β-hydroxy-5,6-dihydrosolasodine obtained from the catalytic reduction of 7β-hydroxysolasodine.[5] Molecular rotation data was also in agreement for a 7β-configuration.

The seemingly contradictory data observed in the hydroxylation of tigogenin (5,6-dihydrodiosgenin) and 5,6-dihydrosolasodine attest to the necessity for more fundamental knowledge concerning the mechanism of microbiological hydroxylation.

Experimental[6]

Microbiological Hydroxylation of 5,6-Dihydrosolasodine.—Erlenmeyer flasks (500-ml.) each containing 200 ml. of corn steep medium,[5] were inoculated with newly formed spores of the fungus *H. piriforme* and agitated on a platform shaker at 29–30° for 67 hr. A solution of 25 mg. of dihydrosolasodine (m.p. 205–208°) in 2.0 ml. of ethanol was added to each flask. The flasks were incubated at 29–30° for 100 hr. The mycelium from the combined flasks was removed by filtration through a thin layer of Celite, and then washed with ethanol. The filtrate was made basic with ammonium hydroxide and extracted with chloroform. Thus in a typical run 1.5 g. of dihydrosolasodine yielded 2.0 g. of light brown, resinous residue, which was shown to consist principally of a lipid material and of three steroidal components by thin layer chromatography (silica gel G, *n*-heptane–ethyl acetate–triethylamine, 2:4:4).

The extract was chromatographed on 50 g. of neutral alumina (grade II, Woelm) with the following eluents: absolute ether, 0.5, 1.0, and 2.0% methanol in ether, finally chloroform. Each fraction was tested by t.l.c. The 0.5% methanol in ether eluate gave 50 mg. of the starting material. The 2% methanol in ether eluate yielded 542 mg. of crude crystalline material which through repeated fractional crystallization from chloroform–ether and from methanol furnished 350 mg. of rhombic prisms: m.p. 221–223°, $[\alpha]^{20}D$ −64.3 ± 2° (*c* 0.86, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.78 and 2.91 μ (OH and NH). It was identical in properties (melting point, mixture melting point, and infrared spectrum) with an authentic specimen of 9α-hydroxydihydrosolasodine (II).

(1) Visiting Scientist (1963–1964), National Institutes of Health.

(2) R. E. Marker, T. Tsukamoto, and D. L. Turner, *J. Am. Chem. Soc.*, **62**, 2525 (1940).

(3) S. Hayakawa and Y. Sato, *J. Org. Chem.*, **28**, 2742 (1963).

(4) L. H. Briggs, R. P. Newbold, and N. E. Stace, *J. Chem. Soc.*, 3 (1942).

(5) Y. Sato and S. Hayakawa, *J. Org. Chem.*, **28**, 2739 (1963).

(6) Melting points were taken on the Kofler block and are uncorrected. Microanalyses were performed by the Microanalytical Services Unit of this laboratory under the direction of Dr. W. C. Alford. The infrared spectra were taken on the Model 21 Perkin-Elmer infrared spectrometer by Mr. H. K. Miller and Mrs. A. H. Wright of this laboratory.

Anal. Calcd. for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51. Found: C, 74.88; H, 10.50.

The **acetate IIa**, prepared in the usual manner (acetic anhydride–pyridine, 1-hr. refluxing) and purified through chromatography (alumina), afforded white prisms: m.p. 197–199°; $[\alpha]^{20}D$ −26.7 ± 1.0° (*c* 1.01, ethanol); $\lambda_{max}^{CS_2}$ 2.77 (OH), 5.75 and 8.07 (3-AcO), and 6.02 μ (N-Ac).

Anal. Calcd. for $C_{31}H_{49}NO_5$: C, 72.19; H, 9.58. Found: C, 72.36; H, 9.64.

The mother liquor, after removal of II, yielded 68 mg. of a substance whose R_f value (t.l.c.) was slightly lower than that of 9α-hydroxydihydrosolasodine and which gave a green coloration on spraying with sulfuric acid (50%). II gave a violet coloration with sulfuric acid. The impure crystals of III were repeatedly crystallized from methanol–ether until homogeniety was achieved as shown by t.l.c. Needles, m.p. 216–219°, $[\alpha]^{20}D$ −37.6 ± 1° (*c* 1.0, $CHCl_3$), were obtained. III analyzed for a monohydroxydihydrosolasodine and agreed in properties (melting point, mixture melting point, and infrared spectrum) with a sample of 7β-hydroxydihydrosolasodine (III, solasodane-3β,7β-diol) prepared by the reduction of 7β-hydroxysolasodine.[5] Molecular rotation difference (ΔMD = MD of III − MD of I) of 102 also agrees well for a 7β-hydroxy-5α-steroid listed as +110.[7]

Anal. Calcd. for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51. Found: C, 75.26; H, 10.73.

The **acetate IIIa** failed to crystallize but t.l.c. and g.l.c. indicated it to be a homogenous product. It analyzed for a O,O,N-triacetyl derivative: $[\alpha]^{20}D$ +7° (*c* 0.578, $CHCl_3$); $\lambda_{max}^{CS_2}$ 5.76 and 8.05 (OAc), and 6.02 μ (N-Ac).

Anal. Calcd. for $C_{33}H_{51}NO_6$: C, 71.06; H, 9.22. Found: C, 70.84; H, 9.52.

Hydrogenation of 9α-Hydroxysolasodine.—A solution of 43 mg. of 9α-hydroxysolasodine[5] in 5 ml. of glacial acetic acid was reduced over 40 mg. of palladium–charcoal (10%) catalyst until slightly more than 1 mmole of hydrogen was absorbed. The product, when chromatographed on alumina (Woelm, grade II) and eluted with 0.5 ~ 2.5% methanol in ether, afforded 5.2 mg. of crystalline substance of m.p. 220–223° from methanol–ether. The properties of this compound were in agreement (melting point mixture melting point, and infrared spectrum) with those obtained from the microbial hydroxylation of 5,6-dihydrosolasodine.

Hydrogenation of 7β-Hydroxysolasodine.—To a solution of 30 mg. of 7β-hydroxysolasodine[5] in 6 ml. of ethyl acetate was added 50 mg. of 10% palladium–charcoal and the mixture was hydrogenated until 1 mole equiv. of hydrogen was absorbed. Thin layer chromatography and infrared spectrum indicated the product to be a mixture of the hydroxy- and ketodihydrosolasodine. The presence of the latter was suspected as due to impure starting material. The crystalline residue was therefore dissolved in 5 ml. of pyridine and reduced with 30 mg. of sodium borohydride. The residue was then submitted to alumina chromatography (Woelm, grade I). The fractions eluted with chloroform yielded needles of m.p. 216–218° from acetone and were identical (melting point and infrared spectrum) with the product from the microbiological hydroxylation.

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The Reaction of Organic Azides with Benzyne

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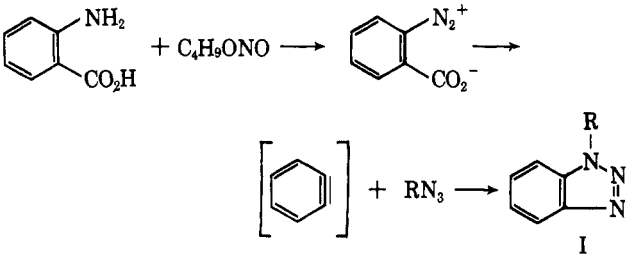
Organic azides react rather sluggishly with olefins and acetylenes to yield triazolines and triazoles.[1] Since the incorporation of the double bond in strained cyclic compounds greatly accelerates the reaction rate, it was thought that benzyne, which contains a strained triple bond, should react readily with azides. This was confirmed several years ago when phenyl azide was treated with benzyne and 1-phenylbenzotriazole was obtained in small yield. The difficulty and danger involved in the preparation of benzyne[2] discouraged further investigation of this reaction.[3] The recent publication of a convenient synthesis for benzyne[4] led to a renewed interest in the problem.

Benzyne has been found to react readily with aromatic, aliphatic, and certain heterocyclic azides. The over-all reaction, including the preparation of the benzyne, is shown in the following reaction sequence. The



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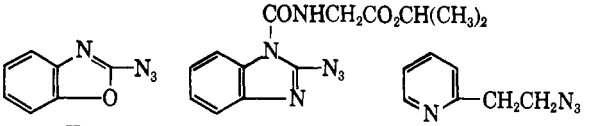
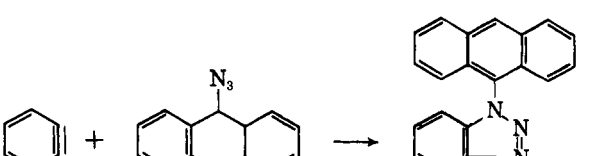
experimental procedure consisted in the slow addition of an acetone solution of anthranilic acid to a refluxing chloroform solution of butyl nitrite and the organic azide, thus eliminating the isolation of the explosive diazobenzoate. The substituted benzotriazoles that were prepared by the procedure are listed in Table I. It is evident that substituents and unsaturation on the aromatic azide do not affect the reaction adversely. It is thought that higher yields of product would be obtained if a metering pump had been used to obtain slower addition of the acetone solution of anthranilic acid. The higher yields of product that were obtained from the aliphatic azides are probably due to better recovery of the product from the reaction mixture.

The results obtained with heterocyclic azides were more complex. The azides II, III, and IV were treated with benzyne by the procedure just described, but the azides were recovered unchanged. It was thought that



II III IV

these azides might not be typical because of interaction between the azide group and the heterocyclic nitrogen atom. The reaction was repeated with 9-azidoacridine (V) which should not be subject to this type of interaction, and 1-(9-acridyl)benzotriazole (VI) was obtained in 47% yield.



V VI

(1) J. H. Boyer and F. C. Canter, *Chem. Rev.*, **54**, 1 (1954).

(2) M. Stiles and R. G. Miller, *J. Am. Chem. Soc.*, **82**, 3802 (1960).

(3) At about this time, Wittig reported that 1-phenylbenzotriazole was formed from phenyl azide and benzyne which was prepared by a different procedure: G. Wittig and R. W. Hoffman, *Angew. Chem.*, **73**, 435 (1961).

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