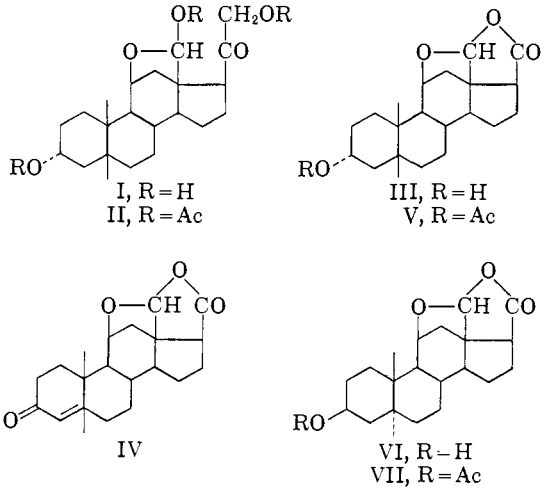
substance, a metabolite of 18-hydroxycorticosterone.[5] We wish to report the correct structure of the aldosterone metabolite as the 3α,5β isomer of tetrahydroaldosterone (I).

Tetrahydroaldosterone (3α,5β) was prepared using a soluble[6,7] enzyme fraction. Male rat livers were homogenized in acetone at −15°, the homogenate was filtered and the residue dried at room temperature in vacuum. The acetone powder was suspended in buffer solution[8] and the filtrate incubated with *d*-aldosterone-21 monoacetate[9] in the presence of TPNH[10] under nitrogen at 37°. Extraction and chromatography of the incubation mixture revealed only one substance (I), other than unchanged substrate, which reduced blue tetrazolium.[11]

Tetrahydroaldosterone (I) was isolated from extracts of combined incubates by chromatography on paper (ethylene dichloride–ethylene glycol, R_{Aldo} = 0.3[12] and on Celite (toluene–ethyl acetate 9:1-methanol:water 1:1, R_{Aldo} = 0.6) as a white amorphous solid, melting range 107–114,°[13] $[\alpha]^{24}D$ + 50° ($CHCl_3$ (C = 0.9), $\lambda_{max}^{CH_2Cl_2}$ 2.78, 5.88 (weak) μ; C, 69.00; H, 8.88. Acetylation with acetic anhydride in pyridine gave a triacetoxy[14] derivative (II) R_{DOCA} = 1.1 in methylcyclohexane–formamide, = 1.3 in methylcyclohexane–methanol:water 4:1, $\lambda_{max}^{CS_2}$ 5.71, 5.77μ, which like aldosterone[15] readily lost one acetoxy group when treated with dilute acetic acid.



Oxidation of tetrahydroaldosterone (I) with periodic acid yielded the theoretical amount of formaldehyde[16] and a monohydroxy γ-lactone (III), m.p. 252–254°, $\lambda_{max}^{CH_2Cl_2}$ 2.88, 5.64 μ, C, 72.46; H, 8.46. This lactone (III) also was obtained from aldosterone etiolactone (IV)[17] using the enzyme preparation described above. Acetylation yielded the monoacetoxy dervative[14] (V), R_{An} = 0.9 in methylcyclohexane–formamide, $\lambda_{max}^{CS_2}$ 5.60, 5.78 μ. There was a single band of simple contour at 8.08 μ which was characteristic of equatorial 3-acetoxy steroids.[18] The 3α,5β configuration was assigned to lacetone III by excluding the other possible 3-equatorial isomer. Hydrogenation of aldosterone etiolactone (IV) over platinum oxide in acetic acid gave the known 3β,5α, lactone VI,[17,19] m.p. 241–243°, which depressed the melting point of lactone III. The infrared spectra of the pair of isomeric lactones (III and VII) and of their acetoxy derivatives (V and VII) differed in the fingerprint region. Only lactone VII formed an insoluble digitonide.

The metabolite of aldosterone (3.8 mg.), isolated as described[4] from the urine (17 day pool) of a patient with cirrhosis of the liver, was compared with tetrahydroaldosterone (3α,5β). Three derivatives (II, III, V) of the isolated and the synthetic steroid were prepared. Both ketols as well as their corresponding derivatives had identical infrared spectra and chromatographic running rates. Both ketols also were shown to be identical by double isotope techniques. Following the administration of *d*-aldosterone-7-H^3, the major radioactive moiety was isolated from the glucuronide fraction of urine and mixed with tetrahydroaldosterone (3α,5β)-4-C^{14}, and derivatives II, III and V of the doubly labeled mixture were prepared. The H^3:C^{14} ratios of the ketol and its derivatives agreed within 5%.

(5) The separation of the metabolite of 18-hydroxycorticosterone from the aldosterone metabolite and the confirmation of its structure by synthesis of the γ-lactone obtained by periodate oxidation will be reported separately, S. Ulick and K. Kusch. In certain states of hyperaldosteronism such as cirrhosis of the liver and maligant hypertension, the secretion of 18-hydroxycorticosterone as measured by the excretion of this metabolite was greater than 4.0 mg. per day, exceeding that of aldosterone.

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(8) *p*H 7.4 containing these concentrations of salts in m. moles per liter: NaCl 110, KCl 4.6, $MgSO_4$ 1.0, Na_2HPO_4 22.

(9) We wish to express our appreciation to Dr. C. H. Sullivan, Ciba Pharmaceutical Products, for providing the *d*-aldosterone-21-monoacetate used in this work.

(10) Generated from TPN (triphosphopyridine nucleotide) by the coupled oxidation of glucose-6-phosphate in the presence of glucose-6-phosphate dehydrogenase.

(11) When cortisol was incubated with the same enzyme preparation, under the same conditions, the only product was 3α,17α,11β,21-tetrahydroxy-20-ketopregnane.

(12) R values refer to the distance migrated by the sample on paper chromatogram relative to the reference steroid. Abbreviations: Aldo = aldosterone, DOCA = desoxycorticosterone acetate, An = 4-androstene-3,17-dione.

(13) Melting points (uncorrected) were determined on a micro hot stage. Only the hydroxyl and carbonyl regions of the infrared are reported here. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories.

(14) The acetate number was determined with H^3-labeled acetic anhydride. The number of moles of steroid present in the sample was determined from its C^{14} content. The c.p.m. C^{14} per mole for tetrahydroaldosterone and its derivative was determined from the specific activity of aldosterone-4-C^{14} used as a substrate in the incubation.

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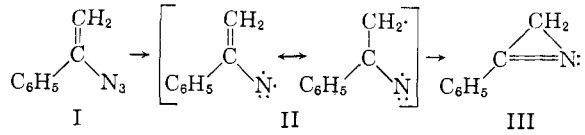
VINYL AZENE CHEMISTRY: FORMATION OF AZACYCLOPROPENE

Sir:

As a continuation of our studies of the chemistry of the monovalent nitrogen species, azenes,[1,2] the

investigation of the decomposition products of vinyl azides was undertaken. Presumably the vapor phase pyrolysis[1c] of α-azidostyrene (I)



I II III

could lead to the formation of styryl azene (II). Of the several conceivable reaction paths open to such an intermediate, it was thought that a likely possibility might be ring closure to give an azacyclopropene. Quite gratifyingly, the pyrolysis of I resulted in a 65% yield of 2-phenylazirine (III).

That III arises by cyclization of II appears quite plausible since any reaction of the double bond of I with the azide moiety would more likely involve the terminal nitrogen atom of the azide leading to a triazole in a manner analogous to tetrazole formation from imino azide derivatives.[3] The ready formation of this novel unsaturated ring system is essentially analogous to the formation of cyclopropenes from alkenylcarbenes.[4]

Although azacyclopropenes have been postulated as intermediates in the Neber reaction,[5] only one such compound, 2-(2,4-dinitrophenyl)-3-methyl-2-azirine, has been isolated and its structure established.[6] Thus the formation of III from I is not only interesting in itself, but represents the first example of what appears to be a useful method for preparing azacyclopropenes—work is now in progress along these lines.

2-Phenylazirine (III) [calcd. for C_8H_7N: C, 82.02; H, 6.02; N, 11.96; mol. wt., 117. Found: C, 81.87; H, 6.23; N, 11.64; mol. wt.,[7] 126] is a colorless, thermally unstable, irritating liquid of b.p. 80° (10 mm.). Its infrared spectrum (CCl_4) shows strong C=N absorption at 5.74 μ[6] and its ultraviolet spectra in cyclohexane and in ethanol show maxima at 239 (13,000) and 242 mμ (13,000), respectively. The n.m.r. spectrum (CCl_4) showed the methylene hydrogens as a sharp line at 8.35τ and the *meta–para* and *ortho* phenyl hydrogens as multiplets in the regions 2.4 and 2.1τ, respectively, with intensity ratios of 2:3:2, respectively. Boiling a solution of III in acidic aqueous ethanol resulted in the formation of 2,5-diphenylpyrazine[8] in 30% yield. These data are consistent with the assigned structure for III.

α-Azidostyrene was prepared as follows: styrene dibromide was treated with one mole of sodium azide in dimethylformamide and the resulting bromoazide dehydrobrominated with potassium tertiary butylate in benzene. The styryl azide was purified by chromatography[1c] [calcd. for $C_8H_7N_3$: C, 66.19; H, 4.86; N, 28.95. Found: C, 65.96; H, 4.87; N, 29.18]. The n.m.r. spectrum (CCl_4) showed the non-equivalent methylene hydrogens as two single sharp lines at 5.68 and 4.68τ and the phenyl hydrogens as a multiplet in the region 2.7τ. The data confirm the structure of I.

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(7) Cryoscopic determination in cyclohexane.

(8) Identity of this material established by comparison with an authentic sample.

THE PHOTOLYSIS OF 2-AMINOPYRIDINES AND 2-PYRIDONES

Sir:

A recent report[1] from these laboratories described the irradiation of 2-aminopyridine hydrochloride with sunlight through Pyrex glass to give a photoisomer with very interesting chemical and physical properties, which was considered to possess the valence-bond tautomeric "Dewar" structure Ia. An analogous photo product (Ib) was obtained from the hydrochloride of 2-amino-5-chloropyridine. Reduction of either Ia or Ib gave the same hydrogenation product, considered to be II. In spite of the evidence which strongly supported these structures, we now wish to report that the photoproducts are, in fact, dimers (IIIa and b).

Conclusive evidence in favor of these revised structures was obtained by alkaline hydrolysis of the tetrahydro derivative IVa (previously considered to be II) to give a product (V) identical in every respect with the tetrahydro derivative of the photodimer (VIa) of 2-pyridone.[2] Since n.m.r. studies on IIIb conclusively exclude the possibility that it could be a 3,4-dimer,[1] the conversion of IV to V constitutes convincing proof that the 2-pyridone photodimer (VIa) (and its N-methyl derivative VIb, to which it has been related by methylation) is also a 3,6- rather than a 3,4- dimer as previously suggested.[3] This conclusion is consistent with the observation that N,6-dimethyl-5,6-dihydro-2-pyridone does not dimerize upon irradiation. Of the four 3,6-structures which can be written for the 2-pyridone dimers, only two (VI and VII) are consistent with the negligible dipole moment previously found for VIb. Of these, VII can be ruled out, since the corresponding structure in the 2-aminopyridine series (VIII) would involve unfavorable proximity of positive charges.

We had previously reported that solution of II (now shown to be IVa) in dilute alkali resulted in the rapid separation of a product which, on the basis of all available evidence, was considered to be *cis*-2-aminocyclobutanecarboxamide (IX). This was not an unreasonable proposal, since II would be expected to undergo a facile hydrolytic ring-cleavage to a less strained system. However, we now have been able to show that this product is actually an extremely stable dihydrate (IVb).

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(3) We would like to express our appreciation to Professor P. deMayo, Department of Chemistry, University of Western Ontario, London, Canada, for revealing to us in advance of publication (*Tetrahedron Letters*, in press) his conclusion that the N-methyl-2-pyridone dimer possesses structure VIb. In point of time, Professor deMayo's conclusion on this revised structure preceded our own, and knowledge of his results greatly facilitated our own investigations.