acetate and water and between *n*-hexane and 3% ethanol. The corticosteroid fraction was subjected to reversed phase partition chromatography on purified acetylated filter paper (Schleicher and Schuell) in the system recently developed,<sup>8</sup> *n*-butyl acetate/23% aqueous ethanol, at 22–25° for 18.5 hours, collecting the overflow for the isolation of aldosterone.

Ultraviolet contact photography of the paper strips showed a spot parallel to corticosterone, but no spots were detected corresponding to cortisone or cortisol; the methanol eluate of the spot gave  $\lambda_{\text{max}}$  at 240 m $\mu$  (EtOH), with an optical density equivalent to 15.1  $\mu g$  of corticosterone An aliquot of the sample reduced blue tetrazolium,<sup>9,10</sup> with a typical color development within 40 minutes. When treated with concd. sulfuric acid at 24° for 2 hours,<sup>11</sup> another aliquot of the sample showed  $\lambda_{max}$  at 287, 375 and 455 m $\mu$  and a shoulder at 330 mµ, and optical density ratios of 1.0/0.30/0.17/0.50. In the Aminco-Bowman spectrophotofluorometer there was a maximum in the activation spectrum at 465 m $\mu$ . Activation at this wave length gave a fluorescent spectrum<sup>12,13</sup> with  $\lambda_{max}$ . at 510 mµ.

The overflow collected from the chromatogram gave a  $\lambda_{max}$ . at 239 mµ(EtOH), equivalent to 54.8  $\mu$ g. of aldosterone. After the fraction had been rerun for 5 hours in the same system, ultraviolet contact photography showed a single intense spot parallel to standard aldosterone with  $R_{\rm f}$  values different from cortisone, cortisol, epicortisol, and  $20\alpha$ - and  $20\beta$ -hydroxycortisol, run on parallel strips. The eluate of the spot from the paper chromatogram gave a  $\lambda_{max}$ . at 239 mµ (EtOH), with an optical density equivalent to 54.6  $\mu$ g. of aldosterone. An aliquot  $(12 \ \mu g.)$  treated with 1 ml. of coned. sulfuric acid<sup>14</sup> at 24° for 2 hours gave  $\lambda_{max}$ . at 288 mµ. After an additional incubation in a water-bath at 90° for one hour the spectrum was characteristically changed, and was identical with that of standard aldosterone eluted from the paper chromatogram, with  $\lambda_{\text{max}}$ . at 245, 285 and 380 m $\mu$  and optical density ratios of 1.0/ 0.99/0.3. When a 2-µg aliquot was treated with blue tetrazolium for 40 minutes,14 it gave a characteristic color, with  $\lambda_{max}$ . at 520 m $\mu$ . Biological assay of the product indicated that it had a sodiumretention activity<sup>15</sup> comparable to that possessed by an authentic sample of dl-aldosterone.

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## THE STRUCTURES OF DELTALINE AND DELPHELINE *Sir:*

We wish to suggest Structure I (I,  $R^1 = -OAc$ ,  $R^2 = -OH$ ) for deltaline and II (II,  $R^1 = -OH$ ,  $R^2 = -H$ ) for delpheline.<sup>1</sup> I and II embody a perhydrophenanthrene skeleton differing slightly but importantly from the ring system proposed for lycoctonine derivatives.<sup>2</sup> We believe that a rearrangement of the Wagner-Meerwein type occurs during acidic hydrolysis of the cyclic acetal groups and converts the perhydrophenanthrene skeleton.<sup>1,2</sup>

The tertiary hydroxyl group of I reacts sluggishly with acetyl chloride to yield *acetyldeltaline* (III,  $R^1 = -OAc$ ,  $R^2 = -OAc$ ),  $C_{29}H_{43}NO_9$ , m.p.  $155-156^{\circ},^{3a}[\alpha]_D^{2\delta} - 31.0^{\circ}$  (CHCl<sub>3</sub>).<sup>3b</sup> Pyrolysis of III at 210-220° and dehydrohalogenation of chloroacetyldelpheline (IV,  $R^1 = -OAc$ ,  $R^2 = -Cl)^1$  in refluxing collidine both gave *dehydrodesoxydeltaline* (V,  $R^1 = -OAc$ ,  $R^2$  absent,  $>C_{13} = C_5 <$ ),  $C_{27}H_{39}NO_7,^{3b}$  m.p. 148.5-150.0°,  $[\alpha]_D^{2j} - 136.0°$  (MeOH),  $\nu_{max}$ . 832 cm.<sup>-1</sup> Catalytic hydrogenation of V over platinum in ethanol gave acetyldelpheline (VI,  $R^1 = -OAc$ ,  $R^2 = -H$ ).<sup>4</sup> The two new routes from deltaline to delpheline strengthen our assumption that no skeletal rearrangement occurred during the previous interconversion.<sup>1</sup>

Saponification of I in refluxing alcoholic potassium hydroxide gave deltamine (VII,  $R^1 = -OH$ ,  $R^2 = -OH$ ),<sup>5</sup>  $C_{25}H_{39}NO_7$ , m.p. 239–240°,  $[\alpha]_D^{33}$ -19.25° (MeOH).<sup>3b</sup> Hydrolysis of the acetal function of VII with hot 10% sulfuric acid yields formaldehyde and demethylenedeltamine (VIII,  $R^1$ = -OH,  $R^2 = -OH$ ,  $R^3 = -OH$ ,  $R^4 = -OH$ ) as the dihydrate,  $C_{24}H_{39}NO_7.2H_2O$ ,<sup>5</sup> m.p. 95–97° (dec.),  $[\alpha]_D^{23} + 27.0°$  (MeOH).<sup>3b</sup> Demethylenedeltamine (VIII) consumed two moles of periodic acid<sup>5</sup> to give a diseco-acid, not isolated, which spontaneously formed a  $\gamma$ -lactone (IX),  $C_{24}H_{35}$ -NO<sub>7</sub>,<sup>3b</sup> m.p. 215.0–216.2°,  $[\alpha]_D^{24} - 64.3°$  (MeOH),  $\nu_{max}$ . 1780 and 1712 cm.<sup>-1</sup>.

 $\gamma$ -Lactonization established the relationship of the tertiary hydroxyl group of I with respect to the carbon atom which eventually becomes a carboxyl during the periodate oxidation of VIII. If no rearrangement had occurred during the acidic genesis of VIII, I would then be expected to possess Structure X ( $\mathbb{R}^1 = -OAc$ ,  $\mathbb{R}^2 = -OH$ ,  $\mathbb{R}^3 - \mathbb{R}^4 =$  $-O-CH_2-O^{-})^{\beta}$  on the basis of the interconversion to desoxylycoctonine. Placement of the OH group at the bridgehead position,  $\mathbb{R}^2$ , in X is not, however, consonant with the chemical behavior of this function. Treatment of deltaline under mild conditions with SOCl<sub>2</sub> gives chloroacetyldelpheline, which reacts nearly instantaneously with ethanolic (1) M. Carmack, J. P. Ferris, J. Harvey, Jr., Phyllis L. Magat, E. W. Martin and D. W. Mayo, Tus JOURNAL **80**, 497 (1958).

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silver nitrate and which undergoes rapid solvolysis in aqueous methanol. The hydroxyl thus exhibits a pattern of reactivity quite unlike that found in the similar bicyclo [4,3,1] decane system of  $\beta$ -caryophyllene alcohol.<sup>7</sup> Furthermore the formation of dehydrodesoxydeltaline (V) under mild conditions $^{6}$  cannot be satisfactorily interpreted with Structure X because the introduction of a double bond at the bridgehead position, R<sup>2</sup>, would require an unacceptable violation of Bredt's rule. Models show, however, that an essentially coplanar bond can fit at the  $C_{13}$ - $C_5$  position of Structure V (no bridgehead). A perhydrophenanthrene skeleton was suggested but not favored by Cookson and Trevett<sup>4b</sup> as a possible precursor to demethylenedelpheline. We believe that the latter and demethylenedeltamine are best represented by XI ( $R^1 = -OH$ ,  $R^2 = -H$ ,  $R^3 =$ -OH,  $R^4 = -OH$ ) and VIII ( $R^1 = -OH$ ,  $R^2 = -OH$ ,  $R^3 = -OH$ ,  $R^4 = -OH$ ), respectively, and that desoxylycoctonine is the Omethyl ether of XI ( $R^1 = -OCH_3$ ).



Figures VIII, X-XI.

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Department of Chemistry Indiana University Bloomington, Indiana Received May 26, 1959

## ENZYMATIC OXIDATION OF N-ACETYLHEXOSAMINES TO N-ACETYLHEXOSAMINIC ACIDS<sup>1</sup>

Sir:

Crude extracts obtained from a strain of *Proteus* vulgaris 31 M contained an enzyme that catalyzed the disappearance of N-acetylglucosamine or Nacetylgalactosamine. Free hexosamines, ketoses or ammonia could not be detected as end-products of this reaction. When purified 250-fold (ammonium sulfate fractionation and chromatography on DEAE-cellulose), enzymatic activity was in-

 $(1)\,$  This work was supported by grants from the U. S. Public Health Service,

dependent of any added cofactor. N-Acetylhexosamine disappearance from the reaction mixture was measured by loss in reducing power<sup>2</sup> or Ly a modification of the Morgan–Elson reaction.<sup>3</sup> Reaction mixtures contained these additions in a final volume of 1 ml.: 1  $\mu$ mole of N-acetylhexosamine, 5  $\mu$ moles of phosphate buffer, *p*H 7.2, and enzyme (50–200  $\mu$ g.). Incubations were carried out by shaking at room temperature and in an air atmosphere for 90 minutes.

It was noted that cne-half mole of oxygen was consumed for each mole of N-acetylhexosamine and reducing sugar that disappeared. The disappearance of substrate was dependent on an aerobic mechanism as shown by a doubling of the rate when the reaction was incubated under oxygen rather than air. Furthermore, incubation under nitrogen completely inhibited N-acetylhexosamine disappearance. These data suggested that the reaction catalyzed by the enzyme involved an oxidation of carbon 1 to yield the corresponding N-acetylhexosaminic acids.

The products (I) of enzyme action on the Nacetylhexosamines were chromatographed on paper in three different solvent systems. Only the  $R_{\rm f}$ values found in a butanol, acetic acid and water system (50:15:25) are reported even though comparable results were obtained with all three systems.  $R_{\rm f}$  values of 0.34 were observed for I when it was adjusted to  $pH \ 8$  prior to spotting and visualized with Cl-starch-KI.<sup>4</sup> Furthermore, I or authentic N-acetylhexosaminic acids, when acidified to pH 1 before applying to the papers, gave spots which reacted readily with the hydroxylamine-FeCl<sub>3</sub> reagents,<sup>5</sup> indicating lactone formation which is characteristic of the N-acetylhexosaminic acids.<sup>6</sup> Hydrolysis of I in 2 N HCl for 2 hours at  $100^{\circ}$  converted I to a compound (II) that reacted with ninhydrin and had an  $R_{\rm f}$  value of 0.22. Synthetic glucosaminic and galactosaminic acids ( $R_{\rm f}$ 0.22) and II behaved identically in three solvent systems. Chromatographically, it was not possible to separate N-acetylglucosaminic acid from Nacetylgalactosaminic acid, nor was it possible to distinguish between glucosaminic acid and galactosaminic acid. These compounds were identified by converting them to their corresponding pentoses.7 The product of N-acetylglucosamine oxidation gave rise to a compound that was identified as arabinose  $(R_f \ 0.26)$ , butanol:ethanol:water, 4:1:1). The product of N-acetylgalactosamine oxidation gave rise to a compound identified as lyxose  $(R_f 0.32, butanol:ethanol:water, 4:1:1)$ . When treated in the same manner as I, authentic samples of the corresponding N-acetylhexosaminic acids and the hexosaminic acids behaved identically.

These data show that the enzymatic reaction catalyzed by *Proteus vulgaris* involves a direct oxidation of the free N-acetylhexosamines to the

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