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,[8] *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 $\lambda_{max.}$ at 240 mμ (EtOH), with an optical density equivalent to 15.1 μg. of corticosterone An aliquot of the sample reduced blue tetrazolium,[9,10] with a typical color development within 40 minutes. When treated with concd. sulfuric acid at 24° for 2 hours,[11] another aliquot of the sample showed $\lambda_{max.}$ at 287, 375 and 455 mμ 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 mμ. Activation at this wave length gave a fluorescent spectrum[12,13] with $\lambda_{max.}$ at 510 mμ.

The overflow collected from the chromatogram gave a $\lambda_{max.}$ at 239 mμ(EtOH), equivalent to 54.8 μg. 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_f values different from cortisone, cortisol, epicortisol, and 20α- and 20β-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 μg. of aldosterone. An aliquot (12 μg.) treated with 1 ml. of concd. sulfuric acid[14] 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 $\lambda_{max.}$ at 245, 285 and 380 mμ 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 mμ. Biological assay of the product indicated that it had a sodium-retention activity[15] comparable to that possessed by an authentic sample of *dl*-aldosterone.

HORMONE RESEARCH LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

H. CARSTENSEN[16]
A. C. J. BURGERS[17]
CHOH HAO LI

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(16) University of Uppsala, Uppsala, Sweden.

(17) Rockefeller Foundation Fellow; on leave from University of Utrecht, Utrecht, Netherlands.

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.[1] I and II embody a perhydrophenanthrene skeleton differing slightly but importantly from the ring system proposed for lycoctonine derivatives.[2] We believe that a rearrangement of the Wagner–Meerwein type occurs during acidic hydrolysis of the cyclic acetal groups and converts the perhydrophenanthrene skeleton into the lycoctonine skeleton.[1,2]

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°,[3a] $[\alpha]_D^{25}$ −31.0° ($CHCl_3$).[3b] 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°, $[\alpha]_D^{25}$ −136.0° (MeOH), $\nu_{max.}$ 832 cm.$^{-1}$ Catalytic hydrogenation of V over platinum in ethanol gave acetyldelpheline (VI, R^1 = —OAc, R^2 = —H).[4] The two new routes from deltaline to delpheline strengthen our assumption that no skeletal rearrangement occurred during the previous interconversion.[1]

Saponification of I in refluxing alcoholic potassium hydroxide gave *deltamine* (VII, R^1 = —OH, R^2 = —OH),[5] $C_{25}H_{39}NO_7$, m.p. 239–240°, $[\alpha]_D^{33}$ −19.25° (MeOH).[3b] 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*, $C_{24}H_{39}NO_7.2H_2O$,[5] m.p. 95–97° (dec.), $[\alpha]_D^{25}$ +27.0° (MeOH).[3b] Demethylenedeltamine (VIII) consumed two moles of periodic acid[5] to give a diseco-acid, not isolated, which spontaneously formed a *γ-lactone* (IX), $C_{24}H_{35}NO_7$,[3b] m.p. 215.0–216.2°, $[\alpha]_D^{24}$ −64.3° (MeOH), $\nu_{max.}$ 1780 and 1712 cm.$^{-1}$.

γ-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 (R^1 = —OAc, R^2 = —OH, R^3—R^4 = —O-CH_2-O-)[6] on the basis of the interconversion to desoxylycoctonine. Placement of the OH group at the bridgehead position, R^2, in X is not, however, consonant with the chemical behavior of this function. Treatment of deltaline under mild conditions with $SOCl_2$ gives chloroacetyldelpheline, which reacts nearly instantaneously with ethanolic

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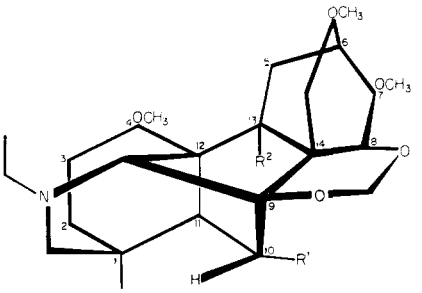
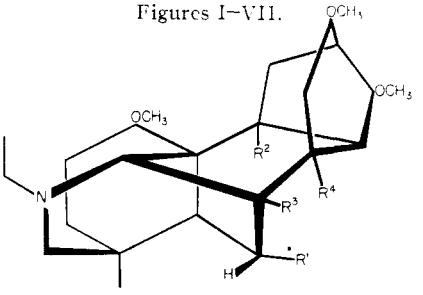
silver nitrate and which undergoes rapid solvolysis in aqueous methanol. The hydroxyl thus exhibits a pattern of reactivity quite unlike that found in the similar bicyclo[4,3,1]decane system of β-caryophyllene alcohol.[7] Furthermore the formation of dehydrodesoxydeltaline (V) under mild conditions[6] cannot be satisfactorily interpreted with Structure X because the introduction of a double bond at the bridgehead position, R^2, 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[4b] 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 O-methyl ether of XI (R^1 = —OCH_3).



Figures I–VII.



Figures VIII, X–XI.

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DEPARTMENT OF CHEMISTRY
INDIANA UNIVERSITY
BLOOMINGTON, INDIANA

MARVIN CARMACK
DANA W. MAYO
JAMES P. FERRIS

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ENZYMATIC OXIDATION OF N-ACETYLHEXOSAMINES TO N-ACETYLHEXOSAMINIC ACIDS[1]

Sir:

Crude extracts obtained from a strain of *Proteus vulgaris* 31 M contained an enzyme that catalyzed the disappearance of N-acetylglucosamine or N-acetylgalactosamine. 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 independent of any added cofactor. N-Acetylhexosamine disappearance from the reaction mixture was measured by loss in reducing power[2] or by a modification of the Morgan–Elson reaction.[3] Reaction mixtures contained these additions in a final volume of 1 ml.: 1 μmole of N-acetylhexosamine, 5 μmoles of phosphate buffer, *p*H 7.2, and enzyme (50–200 μg.). Incubations were carried out by shaking at room temperature and in an air atmosphere for 90 minutes.

It was noted that one-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 N-acetylhexosamines were chromatographed on paper in three different solvent systems. Only the R_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_f values of 0.34 were observed for I when it was adjusted to *p*H 8 prior to spotting and visualized with Cl-starch-KI.[4] Furthermore, I or authentic N-acetylhexosaminic acids, when acidified to *p*H 1 before applying to the papers, gave spots which reacted readily with the hydroxylamine-$FeCl_3$ reagents,[5] indicating lactone formation which is characteristic of the N-acetylhexosaminic acids.[6] Hydrolysis of I in 2 *N* HCl for 2 hours at 100° converted I to a compound (II) that reacted with ninhydrin and had an R_f value of 0.22. Synthetic glucosaminic and galactosaminic acids (R_f 0.22) and II behaved identically in three solvent systems. Chromatographically, it was not possible to separate N-acetylglucosaminic acid from N-acetylgalactosaminic 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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