Compound I (3.00 g., hexahydrate) was allowed to react for 30 minutes with 400 ml. of 0.295 *N* hydrochloric acid at 100 ± 0.5°, and the reaction was then quenched in ice-water containing sodium bicarbonate. The organic material was isolated by continuous extraction with ether, and the unreacted pinacol (1.2 g.) was isolated from the ether solution as the hexahydrate, after addition of a minimum quantity of water. Periodate[1] cleavage of the recovered pinacol afforded acetone-*carbonyl*-C^{14} whose 2,4-dinitrophenylhydrazone (m.p. 125–126°) had a radioactivity assay of 2.240 ± 0.004 mc./mole. The pinacolone remaining in the foregoing ether solution was converted to the 2,4-dinitrophenylhydrazone, m.p. 126–127° (depression when mixed with acetone-2,4-dinitrophenylhydrazone!), radioactivity assay, 4.388 ± 0.012 mc. mole.

Compound II (hexahydrate, m.p. 45–46.5°) was prepared from redistilled biacetyl (b.p. 83–84°) and methyl-C^{14}-magnesium iodide. A repetition of the experiment described above (by which I was subjected to rearrangement) led to the reisolation of 2.1 g. of II-hexahydrate. Cleavage of reisolated II by periodate produced acetone-*methyl*-C^{14} whose 2,4-dinitrophenylhydrazone (m.p. 125–126°) had a radioactivity assay of 2.062 ± 0.001 mc./mole. The pinacolone 2,4-dinitrophenylhydrazone isolated from the same reaction mixture had a m.p. of 126–127°, and a radioactivity assay of 4.122 ± 0.017 mc./mole. From the kinetic data of Duncan and Lynn[4] the conditions for both of the foregoing experiments correspond to values of $a/(a - x)$ in the first-order rate equation of 1.36, or a value of f (fraction reacted) of 0.264. From these data, therefore, it can be calculated[5] that the isotope effects (k^*/k) are, within experimental error of ±1%, for the rearrangement of I, equal to or less than 0.97, for the rearrangement of II, unity.[6]

(4) J. F. Duncan and K. R. Lynn, *J. Chem. Soc.*, 3513 (1956), Table 2.

(5) W. Stevens and R. Attree, *Can. J. Research*, **B27**, 807 (1949); J. Ying-Peh Tong and P. E. Yankwich, *J. Phys. Chem.*, **61**, 540 (1957).

(6) This paper is based upon work performed at Oak Ridge National Laboratory which is operated by Union Carbide Corporation for the Atomic Energy Commission.

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THE ASSOCIATION BEHAVIOR OF β-LACTOGLOBULINS A AND B

Sir:

The discovery of Aschaffenburg and Drewry that β-lactoglobulin consists of two genetically different proteins[1] has led to a reexamination of its electrophoretic heterogeneity.[2,3,4,5,6] Ogston and Tombs[7] found that at *p*H 4.65 β-lactoglobulin A[1] resolves into two peaks on the descending side while the B protein shows only a pronounced skewness of the boundary. From this they concluded that β-lactoglobulin A is primarily responsible for the aggregation between *p*H 3.7 to 5.2,[5,8] while β-lactoglobulin B aggregates to a considerably lesser extent.

Klostergaard and Pasternak[9] reported electrophoretic patterns identical with those of Ogston and Tombs,[7] and also some ultracentrifugal data, with the opposite conclusion that only β-lactoglobulin B associates.

In the course of studies on the molecular behavior of β-lactoglobulin between *p*H 1.5 and 5.5[8,10] we have examined for evidence of aggregation eight samples of β-lactoglobulin A and twelve of β-lactoglobulin B prepared in our laboratory from the milk of individual cows as well as samples of the two proteins kindly given to us by Dr. R. Aschaffenburg. The results obtained showed that all samples of β-lactoglobulin A aggregate strongly at *p*H 4.65 and 2° while none of the samples of β-lactoglobulin B do.

A correlation of the ultracentrifugal and electrophoretic patterns is given in Fig. 1. The ultracentrifugal patterns of the β-lactoglobulin prepared from pooled milk (designated as "normal") and of β-lactoglobulin A exhibits two peaks with $s_{20,w}$ values at 2% protein of 2.8 and 5.3 *S*, corresponding to monomer and aggregate, respectively, while β lactoglobulin B gives a single peak with $s_{20,w}$ of 2.7 *S* for 2% protein. Increasing the protein concentration up to 7% resulted in no evidence of aggregation. The electrophoretic patterns are

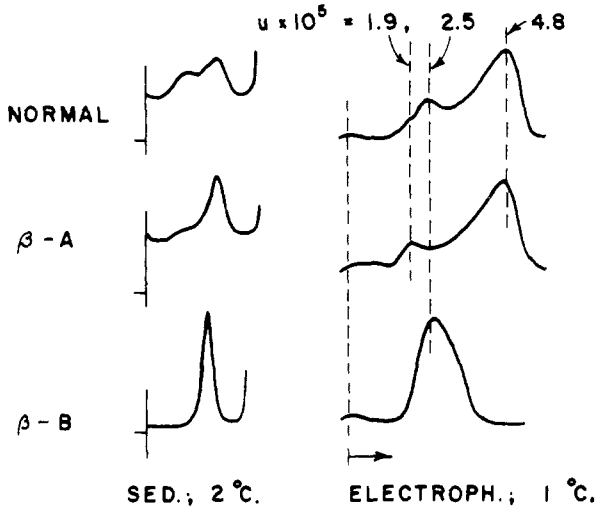

Fig. 1.—Tracings of ultracentrifugal and electrophoretic patterns (descending) of various β-lactoglobulins in *p*H 4.65 acetate buffer, Γ/2 = 0.1. Both sedimentation and electrophoretic migration proceed from left to right: sedimentation, 59,780 r.p.m.; "Normal" and β-A, 1.4% protein, 160 min.; β-B, 7% protein. 352 min.; electrophoresis, 1.6% protein, 8,000 sec. at 9.7 volts/cm.

(1) R. Aschaffenburg and J. Drewry, *Nature*, **176**, 218 (1955); **180**, 376 (1957).

(2) C. H. Li, THIS JOURNAL, **68**, 2746 (1946).

(3) L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid Chem.*, **53**, 126 (1949).

(4) B. D. Polis, H. W. Schmukler, J. H. Custer and T. L. McMeekin, THIS JOURNAL, **72**, 4965 (1950).

(5) A. G. Ogston and J. M. A. Tilley, *Biochem. J.*, **59**, 644 (1955).

(6) S. N. Timasheff, unpublished experiments.

(7) A. G. Ogston and M. P. Tombs, *Biochem. J.*, **66**, 399 (1957).

(8) R. Townend and S. N. Timasheff, *Arch. Biochem. Biophys.*, **63**, 482 (1956).

(9) H. Klostergaard and R. A. Pasternak, THIS JOURNAL, **79**, 5671 (1957).

(10) R. Townend and S. N. Timasheff, *ibid.*, **79**, 3613 (1957).

essentially identical with those reported previously.[7,9] The two peaks observed with β-lactoglobulin A can be identified as monomer (slow) and aggregate (rapid). In the "normal" protein an intermediate peak due to β-lactoglobulin B is also present. The ultracentrifugal and electrophoretic data on β-lactoglobulin A as a function of concentration were analyzed in terms of the Gilbert theory,[11] yielding equilibrium constants for the aggregation in good agreement with those obtained from light scattering.[12,13]

From our ultracentrifugal data we conclude, therefore, that the association of β-lactoglobulin in the *p*H range of 3.7 to 5.2 is due primarily to β-lactoglobulin A, while pure β-lactoglobulin B does not aggregate. This is in direct contradiction of the conclusion of Klostergaard and Pasternak[9] and in agreement with that of Ogston and Tombs.[7] One should remark, however, that the latter reached their conclusion from electrophoretic data alone, which could be open to question in the absence of supporting measurements.

(11) G. A. Gilbert, *Disc. Faraday Soc.*, No. **20**, 68 (1955).

(12) R. Townend and S. N. Timasheff, to be published.

(13) The concentration dependence of Klostergaard and Pasternak's[9] electrophoretic data on β-lactoglobulin A is also strong evidence for association.[11]

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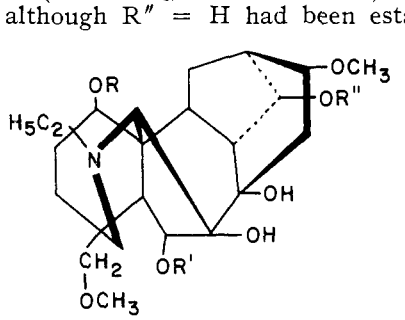
THE INTERRELATION OF DELCOSINE AND DELSOLINE

Sir:

The previous investigation of delcosine (isolated from *Delphinium consolida* L.) had led Anet, Clayton and Marion[1] to assume that the alkaloid possessed the same carbon–nitrogen nucleus as lycoctonine[2] and to suggest for it the tentative structure I (R = CH_3, R′ = R″ = H). In this formula although R″ = H had been established



I

the evidence for R′ = H was meager and rested only on the interpretation of the formation of a carbinolamine ether.[1] The presence of a di-tertiary vicinal glycol has been demonstrated chemically.[3] An attempt now has been made to find further evidence for R′ = H.

(1) R. Anet, D. W. Clayton and L. Marion, *Can. J. Chem.*, **35**, 397 (1957).

(2) M. Przybylska and L. Marion, *ibid.*, **34**, 185 (1956).

(3) R. Anet and L. Marion, *ibid.*, **36**, 766 (1958).

It has been found that diacetyldelcosine,[4] when oxidized with potassium permanganate in acetone gave a lactam, diacetyloxodelcosine, m.p. 103–105°. $[\alpha]^{25}D$ +24.6 ± 1.4° (*c*, 0.71 in chloroform), infrared band at 1644 cm.$^{-1}$ (lactam carbonyl). *Anal.* Calcd. for $C_{28}H_{41}O_{10}N$: C, 60.96; H, 7.49. Found: C, 60.95; H, 7.42. The formation of a lactam shows the presence of a methylene group next to the nitrogen. Hydrolysis of diacetyloxodelcosine with aqueous methanolic potassium hydroxide produced oxodelcosine, m.p. 245–246°, $[\alpha]^{23}D$ +44.3 ± 0.6° (*c*, 1.84 in chloroform), infrared absorption, 1649 and 1622 cm.$^{-1}$ (split lactam band). *Anal.* Calcd. for $C_{24}H_{37}O_8N$: C, 61.65; H, 7.98. Found: C, 61.75; H, 7.98. Oxodelcosine was oxidized by sodium bichromate in acetic acid to a diketonic product, didehydrooxodelcosine, m.p. 211–212°, $[\alpha]^{21}D$ +135.1 ± 0.9° (*c*, 1.11 in chloroform), infrared bands at 3446 cm.$^{-1}$ (OH), 1757 cm.$^{-1}$ (cyclopentanone), 1720 cm.$^{-1}$ (cyclohexanone), 1653 cm.$^{-1}$ (lactam carbonyl); ultraviolet, λ_{max} 297 mμ, log ε 2.07. *Anal.* Calcd. for $C_{24}H_{33}O_8N$: C, 62.19; H, 7.18. Found: C, 62.35; H, 7.12.

The oxidation product is a diketone as expected, but one of the keto groups is in a five-membered ring while the other is in a six-membered ring. According to structure I (R = CH_3, R′ = R″ = H), the oxidation product of oxodelcosine should have contained two cyclopentanone rings. Hence, the substituents cannot be as shown in that structure and, to account for the experimental result, it is necessary to alter the suggested structure of delcosine to I (R = R″ = H, R′ = CH_3). If this structure be the correct one, then delcosine must be very closely related to delsoline which has been assigned structure I (R = H; R′ = R″ = CH_3).[5] Indeed, delsoline should be an O-methyldelcosine.

In order to ascertain this point, delcosine was methylated by means of sodium hydride and methyl iodide in dioxane.[6] The product obtained after chromatography on alumina melted at 215–216° undepressed on admixture with delsoline, and its optical activity, $[\alpha]^{22}D$ +53.4 ± 0.5° (*c*, 2.04 in chloroform) was the same as that of delsoline $[\alpha]^{27}D$ +53.5 ± 0.5° (*c*, 2.11 in chloroform). *Anal.* Calcd. for $C_{25}H_{41}O_7N$: C, 64.22; H, 8.84. Found: C, 64.43; H, 8.79. Its infrared spectrum was superimposable on that of delsoline, and its Debye–Scherrer powder diagram was identical with that of delsoline. The methylation was therefore selective and affected only the secondary hydroxyl located on the five-membered ring. The conversion of delcosine to delsoline thus supports the new structure I (R = R″ = H, R′ = CH_3) suggested for delcosine.

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(4) W. I. Taylor, W. E. Walles and L. Marion, *ibid.*, **32**, 780 (1954).

(5) F. Sparatore, R. Greenhalgh and L. Marion, *Tetrahedron*, in press.

(6) M. Carmack, J. P. Ferris, J. Harvey Jr., P. L. Magat, E. W. Martin and D. W. Mayo, THIS JOURNAL, **80**, 497 (1958), have used this methylation procedure to establish the relationship of deltaline, delpheline and lycoctonine.

(7) National Research Council of Canada Postdoctorate Fellow.