sharp band at 9.8 μ which is reported to be associated with cyclopropyl compounds.[7]

On the basis of the foregoing data compound C appears to be α-amino-2-methylcyclopropanepropionic acid and arises from reduction of the methylene double bond without ring opening. Ring openings at bonds a and b prior to or simultaneous with saturation of the double bond would give rise to the compounds A and B. Splitting at bond c was not observed and is consistent with the observation that methylenecyclopropane undergoes hydrogenolytic cleavage at the bonds adjacent to the unsaturated linkage.[8]

Hypoglycin and its N-acetyl derivatives at 100 mg./kg. caused marked hypoglycemia in fasted rats. The reduced products were inactive.

The increase in liver lipids reported by Chen, *et al.*,[9] also was observed. Altered glucose tolerance and C^{14}-glucose utilization, and inhibition of oxidative phosphorylation by liver mitochondria from hypoglycin-treated rats, along with a decrease in glycogen deposition in rat diaphragms and an increase in R.Q. of liver slices show that the mode of action of this compound differs from that of insulin and further suggests that the hypoglycemic action may, in part, be related to interference with fatty acid utilization.

Detailed accounts of the structural and biological studies will be presented separately in the future.[10,11]

(7) K. Hoffman, O. Jucker, W. R. Miller, G. C. Young, Jr., and F. Tausig, THIS JOURNAL, **76**, 1799 (1954).

(8) J. T. Gragson, K. W. Greenlee, J. M. Derfer and C. E. Boord, *ibid.*, **75**, 3344 (1953).

(9) K. K. Chen, R. C. Anderson, M. C. McGowen and P. N. Harris, *J. Pharmacol. and Exptl. Therapeutics*, **121**, 272 (1957).

(10) We are indebted to H. H. Bird, L. Brancone, W. P. Cekleniak, B. S. Coulomb, J. L. Davis, A. C. Dornbush, W. Fulmor, E. Kaleita, E. L. Markley, H. Siegriest and E. H. Snedeker for assistance in these studies.

(11) Since the submission of this manuscript two papers have appeared supporting structure I for hypoglycin A: C. v. Holt and W. Leppla, *Angew. Chem.*, **70**, 25 (1958), and S. Wilkinson, *Chem. and Ind.*, 17 (1958).

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THE STRUCTURE OF THE CACTUS STEROL LOPHENOL. A LINK IN STEROL BIOGENESIS[1]

Sir:

In contrast to the abundant occurrence of triterpenoid glycosides in giant cacti,[2] the cactus *Lophocereus schottii*[3] was found to be devoid of glycosides but did contain an appreciable neutral fraction. A detailed investigation[4] of it yielded several substances including an unknown alcohol of composition $C_{28}H_{48}O$, which has now been named lophenol. Its structural elucidation has proved to be of considerable biogenetic interest and was aided greatly by the use of three physico-chemical tools—rotatory dispersion, mass spectrometry and vapor phase chromatography—which have been introduced recently into steroid and triterpene work.

Lophenol (I) (m.p. 149–151°, $[\alpha]_D$ +5° (all rotations in $CHCl_3$), *Anal.* found for $C_{28}H_{48}O$: C, 83.82; H, 11.89; O, 3.90) formed an acetate (m.p. 119–121°, $[\alpha]_D$ +28°, *Anal.* found for $C_{30}H_{50}O_2$: C, 81.49; H, 11.10; O, 7.52) and a benzoate (m.p. 161–163°, $[\alpha]_D$ +43°, *Anal.* found for $C_{35}H_{52}O_2$: C, 83.78; H, 10.22). Oxidation with chromium trioxide led to the ketone lophenone (m.p. 122–124°, $\lambda_{max}^{CHCl_3}$ 5.88 μ (6-membered ketone), $[\alpha]_D$ +12°, *Anal.* found for $C_{28}H_{46}O$: C, 84.80; H, 11.31) which regenerated lophenol upon sodium–alcohol as well as $LiAlH_4$ reduction, showing the equatorial nature of the secondary alcoholic function.

The presence of a double bond, typical of Δ^7-unsaturated sterols, was demonstrated by brief treatment with platinum oxide–hydrogen in acetic acid solution to the $\Delta^{8(14)}$-isomer (m.p. 160–163°, $[\alpha]_D$ +19°, *Anal.* found for $C_{28}H_{48}O$: C, 84.04; H, 12.06; acetate, m.p. 79–81°, $[\alpha]_D$ +41°; benzoate, m.p. 140–142°, $[\alpha]_D$ +40°), whose acetate was isomerized with hydrogen chloride–chloroform to the Δ^{14}-acetate (m.p. 133–136°, $[\alpha]_D$ +45°, *Anal.* found for $C_{30}H_{50}O_2$: C, 81.65; H, 11.48; free alcohol, m.p. 156–158°, $[\alpha]_D$ +31°), which could also be obtained directly by hydrogen chloride–chloroform isomerization of lophenol acetate. Hydrogenation of lophenol or of the Δ^{14}-isomer with platinum oxide in acetic acid in the presence of hydrochloric or perchloric acid resulted in saturation of the double bond and formation of lophanol (m.p. 166–168°, $[\alpha]_D$ +27°, *Anal.* found for $C_{28}H_{50}O$: C, 83.49; H, 12.72; acetate, m.p. 131–133°, $[\alpha]_D$ +42°), which was oxidized to lophanone (II) (m.p. 121–123°, $[\alpha]_D$ +25°, *Anal.* found for $C_{28}H_{48}O$: C, 84.29; H, 12.14) and reduced by the Wolff–Kishner procedure to lophane (III) (m.p. 75–77.5°, $[\alpha]_D$ +20°; *Anal.* Calcd. for $C_{28}H_{50}$: C, 86.93; H, 13.13). Further evidence for the presence of a Δ^7-double bond was provided by oxidation of lophenol acetate with perbenzoic acid followed by chromium trioxide[5] to give 7-keto-8,9-oxidolophanyl acetate (IV) (m.p. 190–192°, $[\alpha]_D$ ±0°, *Anal.* found for $C_{30}H_{48}O_4$: C, 75.96; H, 10.35) and 7-keto-8,14-oxidolophanyl acetate (V), (m.p. 175–176°, $[\alpha]_D$ −45°, *Anal.* found for $C_{30}H_{48}O_4$: C, 76.19; H, 10.37; O, 13.54), each of which was converted by ethanolic hydrochloric acid[5] to 7-keto-$^{8(9),14}$-lophadienol acetate (VI) (m.p. 188–190°, $[\alpha]_D$ +3°, λ_{max}^{EtOH} 223.5 and 229 mμ (log ϵ 4.14 and 3.65)).

In spite of this behavior, typical of Δ^7-stenols, the molecular rotation differences of all derivatives in the Δ^7-, $\Delta^{8(14)}$-, Δ^{14}-lophenol and lophanol series were abnormal and incompatible with standard

(1) Supported by grants No. RG-3863 and CY-2919 from the National Institutes of Health of the U. S. Public Health Service.

(2) "Cactus Triterpenes" by C. Djerassi in "Festschrift Arthur Stoll," Birkhauser, Basel, 1957, pp. 330–352.

(3) For occurrence of alkaloids in this cactus see C. Djerassi, S. K. Figdor, J. M. Bobbitt and F. X. Markley, THIS JOURNAL, **79**, 2203 (1957), and earlier papers.

(4) C. Djerassi, G. Krakower, A. J. Lemin, L. H. Liu, J. S. Mills and R. Villotti, to be published.

(5) L. F. Fieser, K. Nakanishi and W. Y. Huang, THIS JOURNAL, **75**, 4719 (1953).

values[6] reported for such sterols. This must have been due to a structural change close to the alcohol function, since the stereochemistry of the ring junctures was clearly of the usual steroid type as demonstrated by rotatory dispersion measurements[7] of the ketones II, IV, V, VI as well as of appropriate Δ^{8}-7-ketones, $\Delta^{8(14)}$-7-ketones and Δ^{14}-16-ketones of the lophenol series, which were all very similar[8] to the dispersion curves of corresponding ketones in the cholestane or ergostane series.

An important clue to this structural change was provided by three observations: (a) None of the above analytical data excluded a C_{27} or C_{29} formula and hence lophane (III) was submitted to mass-spectrographic molecular weight determination[9] which proved a $C_{28}H_{50}$ formula (Calcd. 386.7; found: 386.3 ± 0.6). (b) While the molecular weight determination implied an ergostane side chain (C_9), pyrolysis and vapor phase chromatography of the volatile fragments[10] clearly showed that lophenol possessed a C_8 side chain of the cholestane type, which suggested that an extra methyl group had to be present in the ring. The above described chemical transformations (see formation of VI as well as reformation of I from lophenone with $LiAlH_4$) had already excluded C-5, C-9 and C-14 as possible points of substitution and the abnormal molecular rotation difference calculations suggested proximity to ring A (the presence of the alcohol function at C-3 being assumed by the shape of the rotatory dispersion curve[7] of derived ketones). (c) Exhaustive brominations[11] of lophanone (II) led to ambiguous results, apparently due to spontaneous dehydrobromination and consequent excessive up-take of reagent, but the fact that the rotatory dispersion curve (methanol solution) was unchanged in the presence of hydrochloric acid (in contrast to cholestan-3-one where hemiketal formation is observed) indicated alkyl substitution α to the ketone function (unpublished experiments by L. A. Mitscher). Using the rotatory dispersion curve of II as evidence for the location of the carbonyl group at C-3, these results require the location of a methyl group at C-2 or at C-4. Indeed, lophanone (II) was found by direct comparison (mixture melting point determination, infrared and rotatory dispersion comparison) to be identical with synthetic[12] 4α-methylcholestan-3-one from which it follows that lophenol (I) is 4α-methyl-Δ^{7}-cholesten-3β-ol.

The presence of a cholestane derivative in a plant is noteworthy. Even more striking is the existence of a 4-methylsterol in nature which together with the recently announced structure of cycloeucalenol[13] —a 4-monomethyl triterpenoid—strongly points toward demethylation of squalene cyclization products as a biosynthetic route to plant sterols. Such a course has so far been demonstrated by direct biochemical experimentation only in animals.[14,15]

(6) D. H. R. Barton and W. Klyne, *Chemistry and Industry*, 755 (1948).

(7) For references see C. Djerassi, *Bull. soc. chim. France*, 741 (1957).

(8) The preparation of these ketones and the rotatory dispersion results will be reported in a detailed paper.

(9) P. de Mayo and R. I. Reed, *Chemistry and Industry*, 1481 (1956); we are grateful to Dr. de Mayo for this determination.

(10) This valuable technique for the determination of steroid and terpene side chains has been developed by Prof. E. R. H. Jones and collaborators at Oxford University. We are greatly indebted to them for advance information on this unpublished method and to Dr. L. B. High of Oxford University making out the side chain determination.

(11) This technique has been used successfully in the triterpene series by C. S. Barnes, D. H. R. Barton, A. R. H. Cole, J. S. Fawcett and B. R. Thomas, *J. Chem. Soc.*, 571 (1953).

(12) G. D. Meakins and O. R. Rodig, *ibid.*, 4679 (1956); J. L. Beton, T. G. Halsall, E. R. H. Jones and P. C. Phillips, *ibid.*, 753 (1957). We are indebted to these investigators for a gift of 4α-methylcholestan-3-one.

(13) J. S. G. Cox, F. E. King and T. J. King, *Proc. Chem. Soc.*, 290 (1957).

(14) See F. Gautschi and K. Bloch, THIS JOURNAL, **79**, 684 (1957).

(15) NOTE ADDED IN PROOF.—W. W. Wells and D. H. Neiderhiser, THIS JOURNAL, **79**, 6569 report a sterol from rat feces which is believed to be 4α-methyl-Δ^{7}-cholesten-3β-ol. A mixture melting point of lophenol and of its acetate with that of the sterol and its acetate kindly supplied by Dr. Wells, gave a depression of 4° and 10°, respectively. A small difference in the infrared spectra of the two sterols was observed.

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RACEMIZATION OF LYSINE BY PROTEUS VULGARIS

Sir:

We have discovered that *Proteus vulgaris* ATCC 4669 contains a highly active catalytic system for racemizing lysine. The conversion of L-lysine to DL-lysine by an autolyzed cell suspension of this organism[1] is shown by the data in Table I.

TABLE I

RACEMIZATION OF L-LYSINE BY AN AUTOLYSATE OF *Proteus vulgaris*

Reaction system[a]	μM/ml. Lysine Total[b]	L[c]	D[d]
Complete	21.2	10.5	10.3
Minus *P. vulgaris* cells	21.5	21.7	0.0
Minus L-lysine	0.0	0.0	0.0

[a] Ten ml. reaction mixture containing 35 mg. (dry weight) of washed cells and 220 μM of L-lysine in 0.1 M K_2HPO_4, adjusted to *p*H 8.4 with NH_4OH, was shaken with 0.2 ml. of toluene for 10 minutes and incubated at 28° for four hours. It was brought to *p*H 5.8 with H_3PO_4, held at 100° for 3 minutes, and centrifuged. [b] Quantitative paper chromatography in the following system: methyl ethyl ketone:acetic acid:water (90:25:30 by volume). [c] Manometric assay with L-lysine decarboxylase of *Bacterium cadaveris* NTCC 6578.[2] [d] Quantitative paper chromatography on residual lysine after complete decarboxylation in *c*.

The *p*H optimum of this reaction is 8.4. Similar results are obtainable by directly employing cells in their original culture broth. When a mixture of 10 g. of L-lysine hydrochloride and 100 ml. of fresh culture broth (containing approximately 4 g./l. dry weight of cells), adjusted to *p*H 8.4, was shaken with 2.5 ml. of toluene for 10 minutes and incubated at 28°, racemization was found to be complete in 16 hours. About 80% of the lysine was recovered from the mixture by absorption in IR-120 (NH_4^+ cycle), elution with 4% NH_4OH, and crystallization as the hydrochloride from aqueous ethanol. The product possessed negligible rotation, and was

(1) The medium contained corn steep liquor 20 g., $(NH_4)_2HPO_4$ 10 g., beet molasses 20 g., glycerol 10 g. and $MgSO_4 \cdot 7H_2O$ 1 g., adjusted to *p*H 7.5 with NH_4OH and made up to 1 l. with tap water. Two liters of this medium in a 4 liter fermenter was inoculated with *P. vulgaris* and incubated at 28° with stirring (1750 r.p.m.) and aeration (volume per volume per minute) for 16 hours.

(2) Purchased from Worthington Biochemical Corporation, Freehold, N. J. The assay was carried out as recommended in a one-side-arm flask, with the flask constant corrected for CO_2 retention.