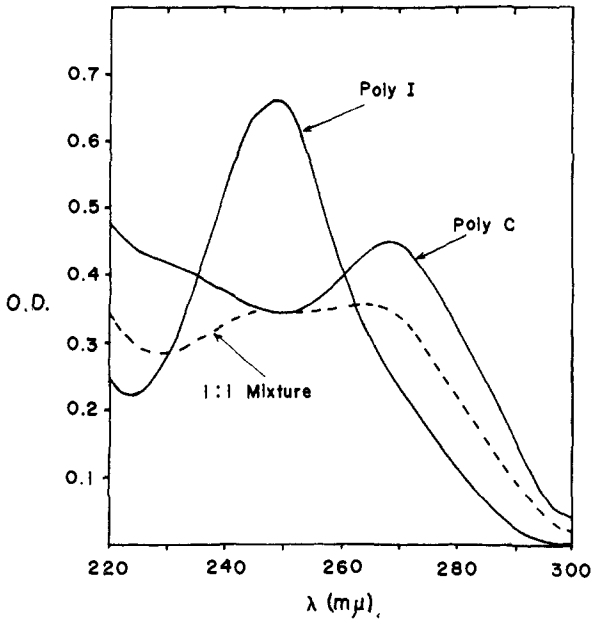

Fig. 1.—The ultraviolet spectra of equal concentrations polyinosinic acid, polycytidylic acid, and a 1:1 mixture of these solutions. All solutions are in 0.1 *M* NaCl, 0.01 *M* sodium cacodylate, *p*H 6.7, $T = 23°$.

which the bases are joined by systematic hydrogen bonding. Models of these helical configurations have been built and their Fourier transforms are being computed for comparison with the observed diffraction data.

We wish to thank Prof. S. Ochoa for the gift of some polynucleotide phosphorylase which was used to prepare the polymers. We are indebted to Mrs. Jean Johnson for technical assistance.

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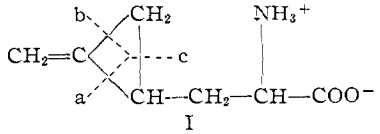
THE STRUCTURE AND BIOLOGICAL ACTIVITIES OF HYPOGLYCIN

Sir:

Hypoglycin A, a substance with hypoglycemic activity, has been isolated from the seeds of *Blighia sapida*.[1,2] It has been described as an amino acid with the empirical formula $C_7H_{11}NO_2$.[3] Work in these laboratories confirms these reported findings and supports structure I for hypoglycin.[4]



I

Hypoglycin is optically active, $[\alpha]^{25}D + 10.3$ (H_2O, *c*. 1.55). Calcd. for $C_7H_{11}NO_2$: C, 59.62; H, 7.86: N, 9.94; mol. wt. 141. Found: C, 59.17; H, 7.81; N, 9.80; Van Slyke N, 11.87; C–CH_3, 0.4%; mol. wt., 139. Acetylation with acetic anhydride in acetic acid or ketene gave two isomeric N-acetyl derivatives: acetylhypoglycin, m.p. 92.5–95.5° (uncor.), $[\alpha]^{25}D + 28.5$ (*c* 1.44, acetone); Calcd. for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.65; 1 C–CH_3, 8.20; mol. wt., 183. Found: C, 58.95; H, 7.42; N, 7.93; neut. equiv., 186; Isoacetylhypoglycin, m.p. 120–121° (uncor.), $[\alpha]^{25}D - 0.08 \pm 0.06$ (*c* 5.3, acetone). Found: C, 58.93; H, 7.19; N, 7.53; C–CH_3, 6.59; mol. wt., 180; neut. equiv., 193.

Lack of any maxima in the ultraviolet indicated the absence of conjugated double bonds. The infrared spectra of hypoglycin, its salts and acetyl derivatives were consistent with an amino acid formulation and displayed bands at 11.25 and 5.70 μ (weak) indicative of a terminal methylene group. Confirmatory evidence for the latter was found in the formation of formaldehyde (10% yield) on ozonolysis of isoacetylhypoglycin and in the formation of a C-methyl on reduction of the double bond (*vide infra*).

Conversion of the acetylhypoglycin into isoacetylhypoglycin by treatment with ketene required a hydrogen atom on the α-carbon and established the epimeric relationship of the isomers. Confirmation of the α-amino acid formulation was obtained by the formation of thiohydantoin and phenylthiohydantoin derivatives.

The failure of D-amino acid oxidase to attack hypoglycin and the ready oxidation by L-amino acid oxidase further confirmed the presence of an α-hydrogen atom and demonstrated the L-configuration of hypoglycin.

The appearance in the nuclear magnetic resonance (n.m.r.) spectrum of potassium isoacetylhypoglycinate in D_2O of a low frequency triplet (1092 cycles) demonstrated the presence of the α-hydrogen of an α-acylamino acid with a β-carbon atom bearing two hydrogen atoms.[5] The formation of aspartic acid by neutral permanganate oxidation of isoacetylhypoglycin, followed by hydrolysis, was consistent with this formulation.

Evidence concerning the three as yet uncharacterized atoms was provided by hydrogenation. Catalytic hydrogenation of hypoglycin in methanol with platinum oxide resulted in the uptake of 1.2 moles of hydrogen per mole. The reduction product was found to be a mixture of three amino acids: A, B and C in the molar proportions of 1:3.5:10. A and B proved to be indistiguishable in the infrared and by paper chromatography from 2-aminoheptanoic acid and 2-amino-4-methylhexanoic acid,[6] respectively.

Compound C (calcd. for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78; 1 C–CH_3, 9.54. Found: C, 58.4; H, 9.16; N, 9.55; C–CH_3, 8.3) was shown to possess a ring by its resistance to further hydrogenation. It lacked the 11.25 μ band but showed a

(1) C. H. Hassall, K. Reyle and P. Feng, *Nature*, **173**, 356 (1954).

(2) C. H. Hassall and K. Reyle, *Biochem. J.*, **60**, 334 (1955).

(3) C. von Holt, W. Leppla, B. Kroner and L. von Holt, *Naturwissenschaften*, **43**, 279 (1956).

(4) As a simplification, we suggest that this new amino acid be referred to as hypoglycin.

(5) Additional peaks were also observed for the terminal methylene (1036 cycles) and the N-acetyl groupings (1177 cycles). The n.m.r. spectrum was taken by Dr. M. Saunders of Yale University on a Varian High Resolution Nuclear Magnetic Resonance Spectrometer at room temperature and 40 mc. The reference scale was based on the absorption of the aromatic and methyl hydrogens of toluene at 1000 and 1197 cycles, respectively.

(6) Both the synthetic and natural samples of the amino acid were probably mixtures of diastereoisomers.

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).