(*Anal.* Found for $C_{19}H_{20}N_2O \cdot HCl$: C, 69.59; H, 6.58; N, 8.52), m.p. 225–226°, which was reduced catalytically to the 3-benzylphthalimidine, then reduced with lithium aluminum hydride to II. II showed an interesting wide spectrum of pharmacological activity. It is a moderately active antihistaminic, a potent local anesthetic, has an antiinflammatory effect in the rat dextran edema test and shows a marked tranquilizing effect on mice.

In exploring variations in the structure of II, compounds obtained by the replacement of the nitrogen of the isoindoline nucleus by carbon, *i.e.*, indenes, were investigated. Such analogs were prepared as follows. 2-(Dimethylaminoethyl)-indane-1-one, (III), b.p. 132° at 1.7 mm. (*Anal.* Found: C, 76.36; H, 8.53; N, 7.12), prepared by a modification of the method by Hoffmann, *et al.*,[1] for similar basic indanones, was treated with Grignard reagents or organolithium compounds. The intermediate amino alcohols dehydrated easily by warming in dilute aqueous acid to give the desired indenes. A large number of compounds were prepared by varying the different structural elements of such indenes. Reaction of III with the organolithium derivative generated by the action of phenyllithium on 2-ethylpyridine and dehydration gave *dl*-2-{1-[2-(2-dimethylaminoethyl)-3-indenyl]-ethyl}-pyridine IV (*Anal.* as the maleate, $C_{21}H_{22}N_2 \cdot C_2H_4O_4$. Found: C, 69.81; H, 6.67; N, 7.16), m.p. 159–161°, the most active antihistaminic of the series.[2] Resolution of IV via the tartrate salts was accomplished. The activity resides chiefly in the levo rotating isomer. This substance as the maleate in the standard *in vivo* guinea pig assay is about four times as potent (oral $ED_{50}$-31$\gamma \pm 3.5\gamma$/kg.)[3] as dextrochloropheniramine maleate, the hitherto most active antihistamine reported.[4]

(1) H. J. Schmid, A. Hunger and K. Hoffmann, *Helv. Chim. Acta*, **39**, 607 (1956).

(2) The generic name is dimethpyrindene.

(3) These compounds were investigated pharmacologically by Drs. W. Barrett and A. Plummer.

(4) F. Roth and W. M. Govier, *J. Pharm. and Exp. Ther.*, **124**, 347 (1958).

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## THE ISOLATION AND CHARACTERIZATION OF THE CHICK EDEMA FACTOR

*Sir:*

The agent responsible for an edema condition in broiler type chicks has been traced[1] to certain lots of feed grade animal fat. This toxic factor has been further restricted[2] to the non-saponifiable portion of the tallow. We wish to report here the isolation of the edema-producing substance in crystalline form and its preliminary characterization.

(1) Anon., *Feedstuffs*, **30**, No. 14, 1 (1958).

(2) S. C. Schmittle, H. M. Edwards and D. Morris, *J. Am. Vet. Med. Assoc.*, **132**, 216 (1958).

A three-week assay involving quantitative measurement of the degree of hydropericardium produced in chicks on a test diet containing the materials to be tested was used to guide the purification of the factor. The details of the biological studies will be reported elsewhere.[3]

The toxic tallow was refluxed in aqueous methanolic sodium hydroxide solution and the neutral, non-saponifiable fraction separated by extraction with ethylene dichloride to yield an active (20×) concentrate.[4] This was dissolved in hexane and chromatographed on alumina (load/adsorbent ratio 1:4). The bulk of the inert material passed through the column and the activity was eluted with 10% ether in hexane. The oily product (500×) was separated from carbonyl-containing impurities by chromatography on Decalso (1:100) using hexane as the developing and eluting solvent. The product (5,000×) was again chromatographed on alumina (1:30) to give a 30,000× concentrate. Finally, this high potency preparation was chromatographed on a high-ratio alumina column (1:1000) using isoöctane as the developing and eluting agent.

The final chromatogram yielded partly crystalline peak fractions. These were triturated with isoöctane to remove oily impurities and the residues were crystallized from a benzene–hexane mixture. After recrystallization to constant properties, the toxic fat factor was obtained as colorless needles which did not melt on the hot stage but sublimed slowly above 225°, rapidly at 245°. It was homogeneous when examined by reverse-phase chromatography on silanated paper. In 1:1 dimethylformamide–isoöctane, a single spot was observed at $R_f$ 0.88. In isoöctane solution, the factor was characterized by absorption maxima at 244 mμ ($E^{1\%}_{1\,cm}$ 1441) and 312 mμ ($E^{1\%}_{1\,cm}$ 117), and a shoulder at 238 mμ ($E^{1\%}_{1\,cm}$ 1138). No absorption maxima were observed in the infrared below 6.3 μ, indicating the absence of carbonyl and hydroxyl groups. In the chick assay, this crystalline toxic factor produced marked hydropericardium at a dose of 0.1 mg./kg. of diet, and was assigned a potency of approximately one million times that of the crude toxic fat.

The small quantity (less than 1 mg.) of crystalline toxic factor isolated to date has not permitted structural studies, even to the extent of elementary analysis. The infrared absorption spectrum is notably lacking in structural implications, but does suggest absence of functional groups containing oxygen. Until the last step in the isolation, it was not possible to correlate any chemical or physical property with the active factor; complete dependence on the chick assay was required.

Other groups studying the toxic principle have used purification methods similar to those outlined here and have suggested that the factor might possess a steroidal[5] or other polynuclear structure.[6,7]

(3) W. H. Ott, *et al.*, to be published.

(4) Activities are expressed as multiples of the toxicity of the original tallow.

(5) W. B. Brew, J. D. Dore, J. H. Benedict, G. C. Potter and E. Sipos, *J. Assoc. Offic. Agr. Chemists*, **42**, no. 1, 120 (1959).

(6) L. Friedman, D. Firestone, W. Horwitz, D. Banes, M. Anstead and G. Shue, *ibid.*, **42**, no. 1, 129 (1959).

(7) J. C. Wootton and J. C. Alexander, *ibid.*, **42**, no. 1, 141 (1959).

Since their preparations, however, were not pure, these structural interpretations must be regarded with reservations. We hope to be able to prepare more pure material shortly for further characterization of this most unusual compound.

We are indebted to Robert W. Walker for the spectral studies. We acknowledge the generosity of B. H. Barrows of the Hales and Hunter Co. and W. B. Brew of the Ralston Purina Co. for supplying us with the fats used in these studies and for helpful discussions of the problem; and we also wish to thank L. Friedman and his associates of the Food and Drug Administration for useful discussions.

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## ISOLATION AND IDENTIFICATION OF D-α-PIPECOLIC ACID, α[L],β-METHYLASPARTIC ACID AND α,β-DIAMINOBUTYRIC ACID FROM THE POLYPEPTIDE ANTIBIOTIC ASPARTOCIN

*Sir:*

The antibiotic Aspartocin produced by the microorganisms *Streptomyces griseus* var. *spiralis* and *Streptomyces violaceus* was isolated recently and characterized as an acidic fatty acid containing polypeptide similar to but different from Amphomycin.[1] Glycine, L-aspartic acid, L-proline and L-valine were reported to have been isolated in crystalline form from a hydrolysate. We now wish to report on the isolation in crystalline form of the other three ninhydrin-positive components and their identification as D-α-pipecolic acid, α[L],-β-methylaspartic acid and α,β-diaminobutyric acid.

Chromatography on Celite with the system 1-butanol, 1-propanol, 0.1 *N* HCl (10:1:10 by volume) gave a band containing pipecolic and β-methylaspartic acids as well as a band of α,β-diaminobutyric acid. The former mixture was resolved by ion exchange chromatography on Dowex 50 × 8 using 1*N* HCl as eluent. Analytically pure pipecolic acid monohydrochloride was obtained by recrystallization from ethanolic ether (Found: C, 43.09; H, 7.20; N, 8.13; $Cl^-$, 21.37; C-Me, none; N-Me, none; calcd. for $C_6H_{11}NO_2 \cdot HCl$: C, 43.38; H, 7.25; N, 8.46; $Cl^-$, 21.15). Optical rotation measurements on the natural compound ($[\alpha]^{25}D$ +15.5°, $C$ = 3.861 in $H_2O$) indicated it to have mainly the D-configuration (reported[2] for the L-form: $[\alpha]^{25}D$ −24.6°, $C$ = 1.087 in $H_2O$). The isolated compound was separated by paper chromatography from β- and γ-pipecolic acids but not from α-pipecolic acid. After racemization[3] matching infrared curves were obtained for the isolated material and authentic DL-α-pipecolic acid.

The methylaspartic acid was recrystallized from aqueous ethanol (Found: C, 40.75; H, 6.41; N, 9.61; C-Me, 7.1; N-Me, 0; $[\alpha]^{25}D$ +12.2°, $C$ = 0.90 in 6 *N* HCl; calcd. for $C_5H_9NO_4$; C, 40.82; H, 6.12; N, 9.52; 1 C-Me, 10.2; reported for α(L),β-methylaspartic acid,[4] $[\alpha]^{27}D$ + 13.3°, $C$ = 3.0 in 5*N* HCl). It was separable by paper chromatography from glutamic acid and α-methylaspartic acid,[5] but not from β-methylaspartic acid.[5] The identity of the isolated compound with α(L),β-methylaspartic acid was supported further by the infrared spectrum.

The α,β-diaminobutyric acid eluted from the Celite column was isolated as the monohydrochloride monohydrate by preparative zone electrophoresis and crystallization from 0.1*N* HCl, pyridine and acetone (Found: C, 27.33; H, 7.59; N, 16.59; $Cl^-$, 20.16; $NH_2$-N, 16.3; ninhydrin $CO_2$, 23.9; C-Me, 5.85; N-Me, 0; $[\alpha]^{25}D$ +16.6°, $C$ = 0.72 in 5*N* HCl; calcd. for $C_4H_{10}N_2O_2 \cdot H_2O \cdot HCl$: C, 27.83; H, 7.54; N, 16.23; $Cl^-$, 20.56; 2 $NH_2$-N, 16.3; 1 ninhydrin $CO_2$, 25.3; 1 C-Me and 1 N-Me, 8.7). The substituted phenylurea was prepared (Found: C, 60.40, 60.49; H, 5.90, 5.59; N, 16.05, 15.83; calcd. for $C_{18}H_{20}N_4O_4$: C, 60.7; H, 5.7; N, 15.7). Of all the structures theoretically possible, only α,β-diaminobutyric and α,β-diaminoisobutyric acids met all the requirements. The latter compound was ruled out on the basis that deamination of the natural product with nitrous acid, then periodate oxidation, yielded not formaldehyde but acetaldehyde as shown by paper chromatography of the 2,4-dinitrophenylhydrazones. The postulated structure, α,β-diaminobutyric acid, was proved by synthesis.[6] The natural and synthetic compounds could not be separated by paper chromatography. The infrared curves showed no differences.

(1) A. J. Shay, J. Adam, J. H. Martin, W. K. Hausmann, P. Shu and N. Bohonos, "Antibiotics Annual 1959/60," in press.

(2) N. Grobbelaar, R. M. Zacharius and F. C. Steward, THIS JOURNAL, **76**, 2912 (1954).

(3) M. Bergmann and L. Zervas, *Biochem. Z.*, **203**, 280 (1928).

(4) H. A. Barker, R. D. Smyth, E. J. Wawszkiewicz, M. N. Lee and R. M. Wilson, *Arch. Biochem. Biophys.*, **78**, 468 (1958).

(5) We are indebted to Dr. H. A. Barker, University of California, for these samples.

(6) C. Kolbe, *J. prakt. Chem.*, [2] **25**, 369 (1882); C. Neuberg, *Biochem. Z.*, **1**, 282 (1906).

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## GLYCEROL: ISOLATION, IDENTIFICATION AND INCORPORATION FACTOR ACTIVITY FOR *B. MEGATHERIUM*

*Sir:*

The purification of a component of bacterial cells and its demonstrated activity in the incorporation of amino acids has been described.[1] We wish to report here studies on a further purified sample of this factor which have led to its identification as glycerol.

Gale and Folkes[1] noted that bacterial cells lose their ability to incorporate amino acids into protein when disrupted and deprived of their nucleic acid. Upon addition of nucleic acid from the same or some other sources, the system was stimulated to resume incorporation of amino acids. Using sonically disrupted and depleted *Staphylococcus aureus* cells for measurement of activity, they

(1) E. F. Gale and J. P. Folkes, *Biochem. J.*, **59**, 661 (1955); **69**, 611 (1958).