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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RECEIVED MARCH 5, 1960

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 $C_6H_{11}NO_2$·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.

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RECEIVED MARCH 7, 1960

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

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