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 $C_6H_{11}NO_2 \cdot HCl$: 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] $[\alpha]^{25}D$ + 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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(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).

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(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).

obtained a highly active "incorporation factor preparation" from nucleic acid. Recently, a simplified assay using starved, intact *Bacillus megatherium* cells was developed by Demain and Newkirk[2] in which ^{14}C-glycine incorporation was also stimulated by the "incorporation factor preparation." The work reported here was accomplished with the guidance of these assays.

Dr. Gale at Cambridge digested a sample of *B. megatherium* nucleic acid with ribonuclease and fractionated it according to the scheme previously described.[1] This incorporation factor preparation was further purified by paper strip chromatography in 10% aqueous sodium bicarbonate. The zone of R_f 0.7–0.9 was eluted with water and the fraction thus obtained separated from colored impurities by passage over ECTEOLA cellulose to give a colorless oil with high incorporation factor activity in both the disrupted *S. aureus* system[3] and the intact *B. megatherium* assay.[2]

Examination of this highly purified incorporation factor[4] showed it to have essentially no ultraviolet absorption or optical rotation. In the paper strip systems previously described, 70% 2-propanol–ammonia and a mixture of ether, alcohol, water, and ammonia 50:40:10:1, it had been reported that the biological activity for disrupted *S. aureus* was located in the areas R_f 0.80 and 0.70, respectively.[1] It has been found possible to locate spots in these areas visually at the γ-level by treating the papers first with periodic acid[5] and then with benzidine acetate. During electrophoresis on paper at *p*H 10.6, incorporation factor moved toward the cathode.[1] However, the rate of movement was found to be identical to that of glucose, and thus was due to electroendosmosis and not to the presence of a positively charged group. No reducing properties could be detected for the periodate-reacting spot. The mobility of the oily incorporation factor and its lack of optical activity suggested a very simple molecule. Assay of a large number of known compounds with the *B. megatherium* assay showed certain sugars, D-gluconic acid delta-lactone and glycerol to possess activity, but glycerol was the most active. In our *B. megatherium* system, glycerol and the purified "incorporation factor" were qualitatively and quantitatively identical in incorporation activity. The paper strip behavior of glycerol was found to be indistinguishable from that of the sample of incorporation factor described above. In addition, the infrared absorption spectra were identical. A crystalline benzoate prepared from incorporation factor was identical with authentic glycerol tribenzoate by m.p. (73–73.5°) and mixed m.p.

The requirement of a cofactor necessary for maximum activity in the assay has been considered, as has also the possibility that in the nucleic acid this incorporation factor exists as a bound form of glycerol with high specific activity. For example, Baddiley and his associates[6] identified cytidine diphosphate glycerol and the teichoic acids as components of bacterial cells. We have tested diacetin, monostearin, sphingomyelin, batyl alcohol, triacetylsphinogosine and other lipids, but none was nearly as active as glycerol itself. Also, beginning with relatively early stages of the fractionation procedure, the reported[1] R_f values of incorporation factor activity are the same as those of glycerol. Whether glycerol exists in a less stable or in a bound form within the nucleic acid remains to be determined.

The authors wish to acknowledge their indebtedness to Dr. Gale both for his many helpful suggestions and discussions, and for supplying them with materials and samples of incorporation factor at various stages of purification.

(2) A. L. Demain and J. Newkirk, to be published.

(3) E. F. Gale, private communication.

(4) Prepared by Dr. Gale in June and studied by us in November, 1959.

(5) H. T. Gordon, W. Thornburg and L. N. Werum, *Anal. Chem.*, **28**, 849 (1956).

(6) J. Baddiley, J. G. Buchanan, A. P. Mathias and A. R. Sanderson, *J. Chem. Soc.*, 4186 (1956).

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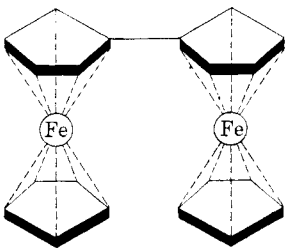
FERROCENE AND RELATED ORGANOMETALLIC π-COMPLEXES. III. AN UNEQUIVOCAL SYNTHESIS OF BIFERROCENYL[1,2]

Sir:

During the course of research in 1957 relating to the synthesis of long-chain trialkylsilylferrocenes from the reaction of ferrocenyllithium and trialkylchlorosilanes, we isolated, in addition to the desired reaction products, trace amounts of a dark-orange, sublimable crystalline solid.[3] This material, after purification by chromatography and recrystallization, melted at 239–240° and had the composition of the dimer of ferrocene, biferrocenyl (I) (Calcd. for $C_{20}H_{18}Fe$: C, 64.91; H, 4.90;



I

Fe, 30.19; mol. wt., 370. Found: C, 65.03; H, 5.08; Fe, 30.39). A definite structural assignment could not be made at that time, however, owing to the lack of reliable molecular weight measurements and to the fact that related homologous ferrocenyl compounds possess nearly identical elemental compositions. Recently, Goldberg and Mayo have suggested the structural assign-

(1) Presented in part at the XVIIth International Congress of Pure and Applied Chemistry, Munich, Germany, August 30–September 6, 1959; Abstract A-147.

(2) Part I, M. D. Rausch, E. O. Fischer and H. Grubert, THIS JOURNAL, **82**, 76 (1960); part II, M. D. Rausch and G. N. Schrauzer, *Chemistry and Industry*, 957 (1959).

(3) Wright Air Development Center Technical Report 57-62, Part II, February, 1958; ASTIA Document No. 150979. This report has been released to the Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C., for sale to the general public.