

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 Newkirk² in which ¹⁴C-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* system³ and the intact *B. megatherium* assay.²

Examination of this highly purified incorporation factor⁴ 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 acid⁵ and then with benzidine acetate. During electrophoresis on paper at pH 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 associates⁶ identified

cytidine diphosphate glycerol and the teichoic acids as components of bacterial cells. We have tested diacetin, monostearin, sphingomyelin, batyl alcohol, triacetylphosphoglycerol and other lipids, but none was nearly as active as glycerol itself. Also, beginning with relatively early stages of the fractionation procedure, the reported¹ 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.

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

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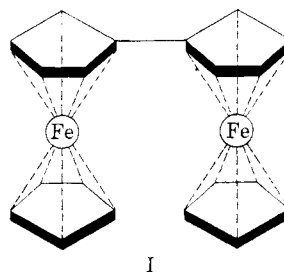
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RECEIVED FEBRUARY 25, 1960

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 C₂₀H₁₈Fe: C, 64.91; H, 4.90;



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.

(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).

ment I for the material on the basis of infrared studies.⁴

We wish to report an unequivocal, convenient synthesis of biferrocenyl by means of the Ullmann reaction of haloferrocenes. For example, the reaction of iodoferrrocene⁵ and activated copper bronze at 150–160° produces biferrocenyl (Found: C, 64.99; H, 4.97; Fe, 30.09; mol. wt., 360), m.p. 239–240° (dec.), in 96–100% yield. The reaction can be carried out under very mild conditions, *viz.*, 60°, without any appreciable lowering in yield of the coupling product. Biferrocenyl prepared in this manner by the well-known Ullmann bi-aryl reaction is identical to biferrocenyl obtained previously, both in melting point and in a comparison of the infrared and ultraviolet spectra of the two products. Examples of quantitative Ullmann bi-aryl couplings are extremely rare in the chemical literature.^{6,7} Moderate to high yields of bi-aryls from the Ullmann reaction usually require activation by strongly electron withdrawing substituents such as the nitro group.

We have also obtained biferrocenyl in reasonably good yield (47–61%), together with ferrocene, from the reaction of diferrocenylmercury^{8,9} and powdered silver at 250–300°.¹⁰ Moreover, the simultaneous heating of an equimolar mixture of diferrocenylmercury and diphenylmercury in the presence of silver has produced phenylferrocene, m.p. 111–112°, in 45% yield, accompanied by lesser amounts of biferrocenyl and ferrocene. Phenylferrocene prepared in this manner was identical to a sample obtained from the reaction of ferrocene and phenyldiazonium chloride.^{11,12} The reaction of diferrocenylmercury with other arylmercury compounds in the presence of silver should constitute an alternate method for the synthesis of arylferrocenes.

Nesmeyanova and Perevalova recently have reported a similar reaction between diferrocenylmercury and palladium black at elevated temperatures.¹³ The yields of biferrocenyl obtained by their method amounted to only 1–6%, however. Nesmeyanov and co-workers also have reported the isolation of biferrocenyl from the reaction of ferrocenylboronic acid with an ammoniacal solution of silver oxide.¹⁴

It is assumed tentatively that the reaction of diferrocenylmercury and silver proceeds via a homolytic mechanism involving ferrocenyl radicals, as has been suggested by Nesmeyanova and

Perevalova in their studies. Experiments designed to elucidate the mechanism and determine the scope of the reaction are in progress.

The discovery of these facile syntheses of biferrocenyl from readily available starting materials^{5,8,9,14} now permits studies of the chemical reactivity of this compound and a comparison of its properties with those of ferrocene. Thus, as in the case of ferrocene,^{15,16} biferrocenyl reacts readily with acetyl chloride and aluminum chloride in the usual Friedel–Crafts manner to produce a mixture of isomeric diacetyl biferrocenyls: red needles, m.p. 147–147.5° (Calcd. for C₂₄H₂₂Fe₂O₂: C, 63.48; H, 4.88; Fe, 24.60. Found: C, 63.32; H, 5.10; Fe, 24.68); red needles, m.p. 191–192° (Found: C, 63.85; H, 4.98; Fe, 24.20). Benzoyl biferrocenyl, m.p. 124–125° (Calcd. for C₂₇H₂₂Fe₂O: C, 68.38; H, 4.68. Found: C, 68.03; H, 4.87), can be prepared by Friedel–Crafts benzoylation of biferrocenyl in a manner analogous to the benzoylation of ferrocene.¹⁷ Studies of this nature and of related reactions of the haloferrocenes are being continued.

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(16) J. H. Richards and T. J. Curphey, *Chemistry and Industry*, 1456 (1956).

(17) M. D. Rausch, M. Vogel and H. Rosenberg, *J. Org. Chem.*, **22**, 903 (1957).

(18) We gratefully acknowledge valuable discussions with Prof. Henry Gilman, Prof. Georg Wittig and Dr. Harold Rosenberg during the course of this investigation.

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RECEIVED MARCH 3, 1960

THE ISOMERIZATION OF BIS-(1-BORACYCLOALKYL) ALKANES VIA THE HYDROBORATION OF DIENES

Sir:

We wish to report that the position of the boron atom in the thermal rearrangement of bis-(1-borocycloalkyl)-alkanes, prepared by the hydroboration of dienes,¹ has been found to be determined by the size of the hetero ring. This result is in contrast to earlier reports² on the isomerization of acyclic secondary or tertiary alkylboranes at 100 to 160° in which migration of the boron atom down a long carbon chain to the terminal position was observed.^{2a}

1,3-Pentadiene was allowed to react with diborane³ (diene: B₂H₆::3:1) at 25° to give a mixture of bis-1,3- and bis-1,4-(1-bora-2-methylcyclopentyl)-pentane (I), which on oxidation with alkaline hydrogen peroxide^{3b} gave an 84% yield of a 10:90 mixture⁴ of 1,3- and 1,4-pentanediols, b.p. 107–114° at 9 mm., *n*_D²⁵ 1.4450, as shown by vapor

(1) R. Köster, *Angew. Chem.*, **71**, 520 (1959); K. A. Saegbarth, to be published.

(2) (a) H. C. Brown and B. C. Subba Rao, *J. Org. Chem.*, **22**, 1137 (1957); *THIS JOURNAL*, **81**, 6434 (1959); (b) G. F. Hennion, *et al.*, *ibid.* **79**, 5190, 5194 (1957); **80**, 617, 3481 (1958).

(3) (a) H. C. Brown and B. C. Subba Rao, *J. Org. Chem.*, **22**, 1136 (1957); (b) *THIS JOURNAL*, **81**, 6428 (1959); (c) H. C. Brown and G. Zweifel, *ibid.*, **81**, 5832 (1959).

(4) Brown and Subba Rao^{3a} report hydroboration-oxidation of pentene-2 yields a 50:50 mixture of 2- and 3-pentanol, thus a 17:83 mixture of 1,3- and 1,4-pentanediol would be predicted on the basis of Structure I.

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(5) A. N. Nesmeyanov, E. G. Perevalova and R. V. Golovnya, *Doklady Akad. Nauk S.S.S.R.*, **99**, 539 (1954).

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(7) P. H. Gore and G. K. Hughes, *J. Chem. Soc.*, 1615 (1959).

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(11) G. D. Broadhead and P. L. Pauson, *J. Chem. Soc.*, 367 (1955).

(12) M. Rosenblum, *THIS JOURNAL*, **81**, 4530 (1959).

(13) O. A. Nesmeyanova and E. G. Perevalova, *Doklady Akad. Nauk S.S.S.R.*, **126**, 1007 (1959).

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