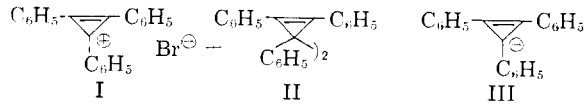
pene systems[1] but of lower intensity. With bromine II is reconverted to 1.6 moles of I, as expected from ionic bromination. The n.m.r. spectrum,[4] with suitable controls, demonstrates the absence of any aliphatic hydrogen; with the bromination results this proves the position of coupling.

Although II is the dimer of the *sym*-triphenylcyclopropenyl radical (III), a solution of II gives no evidence of radicals in the electron spin resonance spectrometer,[4] and the compound is recovered unchanged from several days exposure, in solution, to oxygen or iodine or from two hours reflux with potassium permanganate in pyridine in the pres-



I II III

ence of oxygen (except for 20% conversion to the high melting isomer under these conditions). The dimer is thus remarkably stable with respect to the radical, although III is formally very similar to I, the cation. We take this as further strong evidence for a special stabilization in cyclopropenyl cations which is not shared by either the radical or the anion.

When II is held at its melting point for a few seconds it is quantitatively transformed to an isomer, m.p. 430–432°, which also is formed when II is refluxed in xylene for a few hours or irradiated in solution with an ultraviolet lamp at room temperature. Although we early rejected the possibility that this was hexaphenylbenzene, because of what we now recognize to have been an experimental error, and entertained the idea that this isomer was hexaphenylprismane, careful re-examination establishes that this compound is in fact identical with authentic hexaphenylbenzene.[5] Several possible mechanisms can be formulated for this interesting rearrangement.

(4) We wish to thank Mr. John Martin for the n.m.r., and Mr. Herbert Strauss for the e.s.r., determinations.

(5) W. Dilthey and G. Hurtig, *Ber.*, **67**, 2004 (1934); *cf.* B. Franzus, P. J. Canterino and R. A. Wickliffe, THIS JOURNAL, **81**, 1514 (1959).

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CHEMICAL HYDROXYLATION OF 12a-DEOXYTETRACYCLINE

Sir:

Initial attempts to prepare tetracycline by perbenzoic acid oxidation of 12a-deoxytetracycline were unsuccessful.[1] There was some evidence from ultraviolet data that 12a-hydroxylation occurred but, if so, 12a-epitetracycline must have been formed. Similar results have been reported with perbenzoic acid oxidation of 12a-deoxydedimethylaminotetracycline.[2] Muxfeldt and Kreutzer[3] have recently reported, however, on the preparation of 12a- and 12a-epi-hydroxylated derivatives of 12a-deoxydedimethylamino-anhydrotetracycline-10-monomethyl ether by this method. The encouraging results obtained from the microbiological process[4] prompted attempts to improve the yield of tetracycline via this method. It was thought that by altering the fermentation conditions (*e.g.*, type of medium and time of harvest) an enzyme specific for 12a-hydroxylation might be produced preferentially. While this objective proved unsuccessful, one medium was found capable of 12a-hydroxylation prior to inoculation. The chemical agent responsible for this reaction was found to be sodium nitrite, present as an impurity in the sodium nitrate used in the medium.

Study of the reaction revealed that the optimum *p*H, as in the case of the microbiological process, was from 4.4–4.6. This common optimal *p*H range may reflect the importance of a specific tautomeric form of 12a-deoxytetracycline for optimum reaction rates. The rate of disappearance of 12a-deoxytetracycline, as measured by spectrophotometric assay in 0.1 *M* sodium borate, increased with increasing concentrations of sodium nitrite employed, but at molar ratios of sodium nitrite to 12a-deoxytetracycline of 1 or higher, the yield of tetracycline was reduced. The action of sodium nitrite appears to be in part direct and in part catalytic. Under anaerobic conditions no tetracycline was formed but the 12a-deoxytetracycline and sodium nitrite were consumed in equimolar quantities. Two gases were evolved; carbon dioxide and in much lesser quantity a neutral gas of unknown identity. Aerobically, oxygen was consumed and tetracycline, as indicated by paper chromatography, was produced in .25–35% yield. The catalytic nature of sodium nitrite in the aerobic reaction is suggested by the fact that at low sodium nitrite to 12a-deoxytetracycline ratios (0.05–0.01) considerably more 12a-deoxytetracycline than sodium nitrite was consumed.

For proof of tetracycline formation, 150 ml. of 10 mg./ml. aqueous solution of 12a-deoxytetracycline (*p*H to 1.5 with HCl) was added to 1200 ml. of *p*H 4.4 McIlvaine buffer[5] plus 150 ml. of sodium nitrite solution at 0.808 mg./ml. After two hours of shaking on a reciprocating shaker, spectrophotometric assay indicated 97% consumption of 12a-deoxytetracycline and microbiological assay suggested a 29% yield of tetracycline. The isolation procedure was similar to that previously reported.[4] Three ultraviolet absorbing fractions were obtained by column chromatography. Crystalline tetracycline, as characterized by infrared, ultraviolet, paper chromatography and bioassay was isolated from the most polar fraction (fraction 3). Fraction 1 displayed non-distinctive ultraviolet absorption spectra. However fraction 2 exhibited spectra in 0.1*N* HCl and 0.1*M* sodium borate very similar to those of fraction 2 material obtained from the microbiological reaction.[4] The similarity was further extended by failure to observe anhydro formation upon refluxing with methanolic HCl. It is believed that an 11a-

(1) Unpublished results of Dr. A. Green of these laboratories.

(2) Discussed by C. R. Stephens at the Gordon Research Conference, Medicinal Chemistry, August, 1957.

(3) H. Muxfeldt and A. Kreutzer, *Naturwissenschaften*, **46**, 204 1959).

(4) Holmlund, *et al.*, THIS JOURNAL, **81**, 4750 (1959).

(5) "Handbook of Chemistry and Physics," Chemical Rubber Publishing Co., Cleveland, Ohio, 24th Edition, 1940–1941, p. 1374.

hydroxylated product may also have been produced in the chemical reaction.

Other oxidizing agents also able to 12a-hydroxylate 12a-deoxytetracycline are $KMnO_4$, I_2, $K_2Cr_2O_7$, $K_3Fe(CN)_6$ and $K_2S_2O_8$. Udenfriend's hydroxylation system[6] was inactive. Reducing agents, *e.g.*, ascorbic acid, Na_2SO_3 and $FeSO_4$ inhibited consumption of 12a-deoxytetracycline in both the chemical and microbiological processes.

Grateful acknowledgment is extended to A. Green and J. H. Boothe for supplies of 12a-deoxytetracycline, to A. C. Dornbush for microbiological assays, to J. H. Martin for paper chromatographic assays and to W. Fulmor for infrared analyses.

(6) S. Udenfriend, C. T. Clark, J. Axelrod and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).

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SEPARATION OF NITROGEN AND OXYGEN BY GAS LIQUID PARTITION CHROMATOGRAPHY USING BLOOD AS THE STATIONARY PHASE

Sir:

It has been shown[1,2,3] for a number of olefins that high efficiency of separation of compounds, boiling only 0.1° apart, can be obtained by the use of complex-forming solutions as the stationary phase in gas–liquid partition chromatography.

It appeared that the application of this type of stationary phase could be further extended to the separation of gases of very low solubility, which so far had been considered to be outside the range of gas-liquid partition chromatography. We wish now to report the separation of oxygen and nitrogen by this method.

In order to be useful in partition chromatography, complex-formation must be reversible, and reaction with the substance to be separated must proceed rapidly in both directions. Respiratory pigments fulfill these requirements for oxygen. Accordingly a column was prepared with blood as the stationary phase and, after a number of trials, conditions were found in which the separation of nitrogen and oxygen could in fact be achieved.

Sheep, cow and human blood were used. The animal blood was collected in semi-sterile bottles containing a solution of 3.2% of sodium citrate dihydrate (18 cc. of solution for 100 cc. of blood). Heparin was added to human blood to avoid coagulation.

The solid support was powdered fire brick (Johns–Mansville C-22) of 120–170 or 170–200 mesh. The blood, in the proportion of 0.5 cc. per g. of solid support, was added slowly to the powder while stirring. Mixing was continued until the mass became homogeneous and the free flowing powder was then filled into U shaped glass columns of 4 mm. diameter and 1 m. length. Helium was passed through the columns to desoxygenate the blood and the sample of air (0.4–1.0 cc.) then was injected. When not in use, the columns, filled with helium, were kept in a refrigerator, and could be reëmployed many times. The experiments were carried out with a Perkin Elmer Model 154 A Fractometer.

With a total column length of 2 m. and a temperature of 30–40° excellent separation is obtained, with the oxygen peak being nearly symmetrical. At 20–25° results are less good and no separation occurs at 13°. Also, if the rate of flow is much less than 8 cc. per min., part of the oxygen appears to be bound irreversibly. For a given column and temperature, the retention volume of oxygen will change with the characteristics of the blood, since the partition coefficient will vary with such factors, as the percentage of hemoglobin, and the affinity of hemoglobin for oxygen in the environmental conditions (*p*H, concentration of hemoglobin in the erythrocytes, etc.). Thus, *e.g.*, blood of patients having various blood diseases, and, in particular, different hemoglobin percentages and erythrocyte counts, gave different values for the retention volume of oxygen in the same chromatographic conditions (work of the authors with D. Dannon and L. R. Rosenstein). It is to be noted that the partial pressure of oxygen in the chromatographic column is of the order of a few mm. only, that is, the uptake and release of oxygen proceeds at quite different pressures than in the living organism and the degree of saturation of the hemoglobin at equilibrium is low.

Work is in progress to examine the possible applications of these findings and to extend the method to the analysis of substances other than oxygen (*e.g.*, carbon monoxide) and to complex-forming compounds other than respiratory pigments.

(1) B. W. Bradford, D. Harvey and D. E. Chalkley, *J. Inst. Petrol.*, **41**, 80 (1955).

(2) E. Gil-Av, J. Herling and J. Shabtai, *J. of Chromatog.*, **1**, 508 (1958).

(3) J. Shabtai, J. Herling and E. Gil-Av, *J. of Chromatog.*, in press (1959).

(4) Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Massachusetts, on sabbatical leave from the Weizmann Institute of Science, Rehovoth, Israel.

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IDENTITY OF THE α CHAINS OF HEMOGLOBINS A AND F

Sir:

Possible identity of portions of human fetal and adult hemoglobin was suggested by Schroeder and Matsuda,[1] who determined that fetal, like adult,[2] hemoglobin contained two polypeptide chains N-terminal in the sequence val-leu (α chains).[3] This suggested identity now has been substantiated by our present experiments which show not only that "fingerprints"[5] of the soluble portion of tryptic

(1) W. A. Schroeder and G. Matsuda, THIS JOURNAL, **80**, 1521 (1958).

(2) H. S. Rhinesmith, W. A. Schroeder and L. Pauling, *ibid.*, **79**, 4682 (1957).

(3) The two γ chains of fetal hemoglobin terminate in glycine[1] and the two β chains of adult hemoglobin in val-his-leu.[4]

(4) H. S. Rhinesmith, W. A. Schroeder and N. Martin, THIS JOURNAL, **80**, 3158 (1958).

(5) V. M. Ingram, *Biochim. Biophys. Acta*, **28**, 539 (1958).