NOTES.

749. *Biosynthesis of Polynucleotides. Part III.* Selective Phosphorylation of Adenosine.*

By G. R. Barker and G. E. Foll.

Early attempts to use phosphorus oxychloride for the phosphorylation of adenosine did not result in useful preparative methods.[1,2] The low yields obtained were no doubt due partly to the inadequacy of the methods then available for isolation of the products. Although mixed nucleotides could not then be quantitatively analysed, the results suggested that the position of phosphorylation of unprotected adenosine depended on the conditions used: in dry pyridine, adenosine-5′ phosphate was believed to be formed,[1] whereas what is now presumed to have been a mixture of adenosine-2′ and -3′ phosphate was produced in aqueous barium hydroxide.[2]

The crude product obtained by interaction of adenosine and phosphorus oxychloride in presence of dry pyridine has now been chromatographed on Dowex-1 formate resin, elution being by 0·15M-formic acid.[3] Fractions corresponding to adenosine-5′, -2′, and

* Part II, preceding paper.

[1] Jachimowicz, *Biochem. Z.*, 1937, **292**, 356.
[2] Gulland and Barker, *J.*, 1942, 231.
[3] Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 1471.

-3′ phosphate were obtained (and identified by paper chromatography) in molecular proportions of 6 : 1 : 3.

Phosphorylation with 1 mol. of phosphorus oxychloride and pyridine containing 0·1 mol. of water gave different results. In various experiments the addition of water followed or preceded the addition of phosphorus oxychloride, but in all cases chromatography of the crude product yielded the 5′-, 2′-, and 3′-phosphate in appreciable quantities, the molecular proportions being 1·5 : 1·75 : 1 and 1 : 2·7 : 2·5 under different experimental conditions.

Ion-exchange chromatography of the crude product obtained by phosphorylation in presence of aqueous barium hydroxide as previously described [2] yielded only two fractions. Under the conditions used, adenosine-5′, -2′, and -3′ phosphates are readily eluted separately in that order,[3] and since it is unlikely that the 2′-phosphate would be produced without any 3′-phosphate or *vice versa*, the appearance of only two fractions suggested the absence of the 5′-phosphate. This was confirmed by the fact that the mixed product suffered no dephosphorylation by the 5′-nucleotidase of Russell's viper's venom.[4] The molecular proportions of the 2′- and 3′-phosphate, calculated from the elution diagrams, were 6 : 4.

It is thus confirmed that whereas in dry pyridine phosphorus oxychloride reacts preferentially with the primary hydroxyl group of adenosine, in aqueous barium hydroxide this hydroxyl group is not attacked; the use of moist pyridine gives results intermediate between the two extremes. In no experiment did the yield of nucleotide justify the use of the method preparatively. Viscontini and Bonetti [5] have pointed out that in the reaction of phosphorus oxychloride with water, the first compound to be formed is $HO{\cdot}POCl_2$, and, although no cyclic ester was recognised in the present experiments, it seems reasonable to assume that the formation of 2′- and 3′-phosphate in the presence of water is due to the reaction of the above dichloride with the possible initial formation of a cyclic phosphate. In dry pyridine, more complex products may be produced first [6] and these are less likely to be formed by reaction at the secondary hydroxyl groups for steric reasons.[7] Analogies with these selective phosphorylations are being sought among biological phosphorylations of nucleosides.

Experimental.—Phosphorylations in pyridine. To adenosine (dried at 100°/0·01 mm. for 24 hr.) (0·1 g.) in pyridine (dried with potassium hydroxide and by distillation twice from phosphoric oxide) (5 c.c.) at 0°, phosphorus oxychloride (redistilled immediately before use) (0·075 c.c.) in dry pyridine (0·5 c.c.) was added dropwise with stirring in the absence of atmospheric moisture. In some experiments, water (0·0075 c.c.) in pyridine (0·5 c.c.) was added either before or after the phosphorus oxychloride. The solution was set aside at room temperature overnight, then cooled to −10°, and 50% aqueous pyridine was added. N-Sodium hydroxide equivalent to the chloride ions present was added and the solvent removed under reduced pressure. The residue was dissolved in water and examined by paper chromatography. The bulk of the solution was brought to pH 8, diluted to give an approximately 0·05M-solution with respect to phosphorus, and percolated through a column (5 × 15 cm.) of Dowex-1 formate. The column was eluted as described by Cohn,[3] appropriate fractions were combined, and the concentration of nucleotide was determined spectroscopically. The solvent was removed under reduced pressure and finally by freeze-drying. Each fraction was identified by paper chromatography (Carter's method [8]). The yield of adenosine-5′ phosphate amounted to 4% in the experiments with dry pyridine.

Phosphorylation in aqueous barium hydroxide. Hydrated barium hydroxide (30 g.) was dissolved in hot water (25 c.c.), adenosine (1 g.) was added, and the solution vigorously shaken

[4] Gulland and Jackson, *Biochem. J.*, 1938, **32**, 597.
[5] Viscontini and Bonetti, *Helv. Chim. Acta*, 1951, **34**, 2435.
[6] Forrest and Todd, *J.*, 1950, 3295.
[7] Barker and Parsons, *Chem. and Ind.*, 1955, 1009; Barker, Montague, Moss, and Parsons, *J.*, 1957, 3786.
[8] Carter, *J. Amer. Chem. Soc.*, 1950, **72**, 1466.

and cooled to 0°. Freshly distilled phosphorus oxychloride (1 c.c.) was added and shaking was continued, the temperature being kept below 10°. Two further quantities (1 c.c.) of phosphorus oxychloride were added, the temperature being brought to 0° before each addition. The mixture was diluted with water and freed from barium ions by adding N-sulphuric acid. Chloride ions were removed from the filtrate by addition of silver acetate and filtration, and the filtrate was adjusted to approximately pH 8 with 2N-sodium hydroxide. An aliquot part (75 c.c.) of the solution (1130 c.c.) was percolated through a column (1 × 10 cm.) of Dowex-1 formate and eluted as in the previous experiment, the two fractions being identified by paper chromatography. The yield of mixed nucleotides calculated from the spectroscopic measurements amounted to 14%. A further aliquot part (2 c.c.) was subjected to paper electrophoresis at pH 3·5 (citrate buffer) and 600 v. The paper was dried and examined in ultraviolet light.[9] A single spot was obtained and was eluted from the paper with water (3 × 10 c.c. at 90°). The combined eluates were concentrated under reduced pressure to 1 c.c. brought to pH 9 with 0·01N-sodium hydroxide, and incubated for 17 hr. at 37° with Russell's viper's venom (Ross Allen's Reptile Institute, Silver Spring, Florida) (1 mg.). The solution was then transferred to paper and subjected to electrophoresis as before. A single spot was again obtained and no material was observed in the region of the starting-line, indicating the absence of nucleoside.

THE UNIVERSITY, MANCHESTER. [*Received, October 1st*, 1956.]

[9] Holiday and Johnson, *Nature*, 1949, **163**, 216.

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750. *Methyl 2-Oxohexacosanoate.*

By N. G. BOAST and N. POLGAR.

IN connection with another investigation the keto-ester named above has been prepared by conversion of hexacosanoic acid into methyl 2-hydroxyhexacosanoate, and oxidation of the latter with chromium trioxide in glacial acetic acid.

The oxidation of methyl 2-oxohexacosanoate, by means of potassium permanganate in acetone, to pentacosanoic acid is also described.

Experimental.—Infrared spectra were determined for Nujol mulls.

Hexacosanoic acid. 13-Oxohexacosanoic acid was obtained from methyl 3-oxohexadecanoate [1] and methyl 11-iodoundecanoate (prepared as described [2] for the preparation of the corresponding ethyl ester) according to the general procedure given by Ställberg-Stenhagen and Stenhagen.[2] The keto-acid had m. p. 99·5° after crystallisation from glacial acetic acid (Found: C, 76·0; H, 12·2. Calc. for $C_{26}H_{50}O_3$: C, 76·0; H, 12·3%) (Stenhagen and von Sydow [3] record m. p. 98·1—98·3°). Reduction by refluxing the keto-acid (10·2 g.) with potassium hydroxide (17 g.), 90% hydrazine hydrate (19 c.c.), and triethylene glycol (52 c.c.) for 3·5 hr., followed by removal of water (until the temperature reached 198°), and further refluxing for 5 hr., gave on acidification hexacosanoic acid, m. p. 88° after crystallisation successively from benzene and glacial acetic acid (Found: C, 78·7; H, 13·3. Calc. for $C_{26}H_{52}O_2$: C, 78·7; H, 13·2%). Stenhagen and von Sydow [3] give m. p. 87·5°.

Methyl 2-hydroxyhexacosanoate. The procedure given by Mendel and Coops [4] for the lower homologues was followed. The crude product was chromatographed in light petroleum (b. p. 60—80°)–benzene (3 : 1) on alumina (activity I; Brockmann and Schodder [5]). Benzene–ether eluted *methyl 2-hydroxyhexacosanoate*, m. p. 79° (from ethanol) (Found: C, 76·3; H, 12·7. $C_{27}H_{54}O_3$ requires C, 76·0; H, 12·7%). Infrared absorption: bands at 3436 (OH) and 1721 cm.$^{-1}$ (CO).

Methyl 2-oxohexacosanoate. The hydroxy-ester (433 mg.) in glacial acetic acid (9 c.c.) was heated with a solution of chromium trioxide (77·5 mg.) in 50% aqueous acetic acid (1 c.c.) at 65° for 0·5 hr., then poured into water. The resulting *keto-ester* had m. p. 73° (from methanol)

[1] Ställberg-Stenhagen, *Arkiv Kemi, Mineralog. Geol.*, 1945, **20**, *A*, No. 19.
[2] Ställberg-Stenhagen and Stenhagen, *ibid.*, 1944, **19**, *A*, No. 1.
[3] Stenhagen and von Sydow, *Arkiv Kemi*, 1953, **6**, No. 29.
[4] Mendel and Coops, *Rec. Trav. chim.*, 1939, **58**, 1133.
[5] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

(Found: C, 76·0; H, 12·3. $C_{27}H_{52}O_3$ requires C, 76·3; H, 12·3%); its infrared spectrum showed carbonyl bands at 1742 and 1730 cm.$^{-1}$. The *oxime*, prepared by refluxing the keto-ester (0·05 g.) in benzene (0·5 c.c.) with hydroxylamine hydrochloride (0·1 g.) and sodium acetate (0·2 g.) in aqueous ethanol for 35 hr., had m. p. 101° [from light petroleum (b. p. 80—100°)] (Found: C, 74·1; H, 12·1; N, 3·6. $C_{27}H_{53}O_3N$ requires C, 73·8; H, 12·1; N, 3·2%).

Oxidation of the keto-ester. Powdered potassium permanganate was added to a boiling solution of methyl 2-oxohexacosanoate in dry acetone in small amounts at 10 min. intervals until the rate of oxidation became very slow. The product was pentacosanoic acid, m. p. 81·5° (from acetone) (Found: C, 78·3; H, 13·3. Calc. for $C_{25}H_{50}O_2$: C, 78·5; H, 13·2%).

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751. *Some Methyl Ethers of Diosgenin.*

By W. J. PEAL.

THE preparation of the methyl 3 : 5-*cyclo*ethers of the sterols is one of the standard methods employed for the protection of these substances at the 3β-hydroxyl group and the 5 : 6-double bond during further reaction.[1,2,3]

Diosgenin toluene-*p*-sulphonate, when refluxed with dry methyl alcohol and anhydrous potassium acetate, gave 3 : 5-*cyclo*diosgenin methyl ether (6β-methoxy-3 : 5-*cyclo*-25*D*-spirostan), isolated as a gum, together with the normal methyl ether. The former ether was characterised by conversion into diosgenin chloride, diosgenin acetate, and diosgenin by standard methods.[2,3,4]

The molecular-rotation difference ($[M]_D$ of methyl 3 : 5-*cyclo*ether—$[M]_D$ of 3β-hydroxy-steroid) of +351° is in fair agreement with the figure of +364° obtained for various sterols.[2]

The normal methyl ether was prepared by refluxing diosgenin toluene-*p*-sulphonate with methyl alcohol and, secondly, by treating the potassium derivative of diosgenin with methyl iodide.

Experimental.—Light petroleum had b. p. 60—90°. $[\alpha]_D$ are in $CHCl_3$.

Diosgenin toluene-p-*sulphonate.* Diosgenin (3 g.) and toluene-*p*-sulphonyl chloride (3 g.) were left in dry pyridine (15 ml.) overnight. After dilution with water the product was extracted 3 times with ether, and the extract washed with dilute hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and concentrated, giving a gum which, after 3 crystallisations from acetone, had m. p. 155·5—156·5° (decomp.) or 158·5—159·5° (decomp.) (depending on rate of heating), $[\alpha]_D^{25}$ −94·0° (*c* 1·0) (Found: C, 71·7; H, 8·4. $C_{34}H_{48}O_5S$ requires C, 71·8; H, 8·5%).

Diosgenin methyl ether. (1) Diosgenin toluene-*p*-sulphonate (2·0 g.) was refluxed in methyl alcohol (200 ml.) for 2 hr. The solution was concentrated to 80 ml. and allowed to crystallise. Diosgenin methyl ether (m. p. 180—182°; 1·6 g.) was obtained which after two recrystallisations from methyl alcohol, had m. p. 180—182°, $[\alpha]_D^{25}$ −119° (*c* 0·5) (Found: C, 78·8; H, 10·3; OMe, 6·8. $C_{28}H_{44}O_3$ requires C, 78·5; H, 10·4; OMe, 7·25%).

(2) Diosgenin (1·0 g.) in dry benzene (30 ml.) was added to potassium (0·25 g.) emulsified under dry benzene (15 ml.). Methyl iodide (4 ml.) was added, the mixture refluxed for 4 hr., then methyl alcohol (1 ml.) added. The benzene solution was washed several times with water, dried (Na_2SO_4), and concentrated. The residual solid was chromatographed on alumina from light petroleum. Elution with light petroleum and light petroleum–benzene (20 : 1), and recrystallisation from methyl alcohol gave diosgenin methyl ether (0·75 g.), m. p. 180—182° (alone or mixed with the previous product), $[\alpha]_D^{25}$ −114° (*c* 1·2).

3 : 5-cyclo*Diosgenin methyl ether.* The toluene-*p*-sulphonate (3 g.) was refluxed for 6 hr. in dry methyl alcohol (300 ml.) containing anhydrous potassium acetate (3 g.). After methyl alcohol (200 ml.) had been distilled off under reduced pressure the solution was diluted with

[1] Fernholz and Ruigh, *J. Amer. Chem. Soc.*, 1940, **62**, 3346.
[2] Hey, Honeyman, and Peal, *J.*, 1952, 4836.
[3] Beynon, Heilbron, and Spring, *J.*, 1936, 907.
[4] Rees and Shoppee, *J.*, 1954, 3422.

water and extracted with benzene. Concentration of the benzene extracts gave a gum (1·80 g.) which was chromatographed on alumina (60 g.) from light petroleum. Elution with light petroleum (5 × 100 ml.) gave a gum (0·79 g.), which after two recrystallisations from acetone had m. p. 88—89°, $[\alpha]_D^{25}$ −35° (*c* 0·6) (Found: C, 78·5; H, 10·4; OMe, 6·6. $C_{28}H_{44}O_3$ requires C, 78·5; H, 10·4; OMe, 7·25%). This substance is considered to be 3 : 5-cyclo*diosgenin methyl ether* from its specific rotation and from the rearrangement given below. Elution of the column with benzene–light petroleum (1 : 5) gave diosgenin methyl ether (0·09 g.) identified by m. p. and mixed m. p. Elution with benzene and benzene–chloroform mixtures gave unchanged diosgenin (0·20 g.), identified similarly.

Rearrangements of 3 : 5-cyclo*diosgenin methyl ether.* (*a*) The 3 : 5-*cyclo*ether (0·26 g.) was dissolved in acetic acid (10 ml.), and concentrated hydrochloric acid (0·4 ml.) was added. Needles of *diosgenin chloride* (0·21 g.) began to separate at once. After three recrystallisations from methyl alcohol–ethyl acetate they had m. p. 213—216°, $[\alpha]_D^{25}$ −109° (*c* 0·5) (Found: C, 74·4; H, 9·5; Cl, 8·6. $C_{27}H_{41}O_2Cl$ requires C, 74·8; H, 9·6; Cl, 8·2%). There was no depression (m. p. 214—216°) of mixed melting point with a sample prepared by the action of thionyl chloride on diosgenin.

(*b*) The 3 : 5-*cyclo*ether (0·18 g.) was refluxed for 5 hr. in acetic acid (10 ml.) containing freshly fused zinc acetate (1·0 g.). The acid solution was diluted and the product (0·18 g.) isolated in the usual way. After two recrystallisations from methyl alcohol–ethyl acetate the diosgenin acetate had m. p. and mixed m. p. 195—198°.

(*c*) The 3 : 5-*cyclo*ether (0·46 g.) in dioxan (50 ml.), water (20 ml.), and 2N-sulphuric acid (1 ml.) was left overnight, the mixture concentrated to 20 ml., and diluted with water, and the crude product was isolated in the usual way. Purification was by chromatography on alumina from benzene. Elution with benzene–chloroform (50 : 1) gave diosgenin (0·27 g.), m. p. and mixed m. p. 206—209° (from methyl alcohol–ethyl acetate).

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MAKERERE COLLEGE, KAMPALA, UGANDA. [*Received, February 18th,* 1957.]

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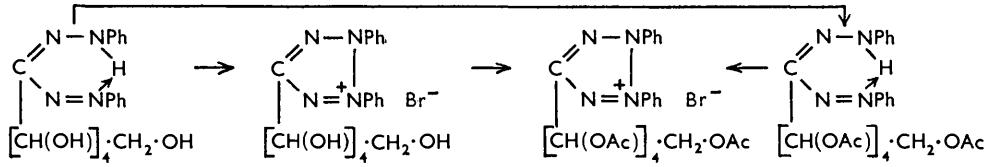
752. *Direct Conversion of Sugar Formazans into Tetrazolium Compounds.*

By L. MESTER and A. MESSMER.

TETRAZOLIUM derivatives of sugars have so far been prepared only by oxidation of acetylated sugar formazans and hydrolysis of the acetylated tetrazolium compounds so obtained.[1] Oxidation of the free sugar formazans with hydrochloric acid and pentyl nitrite or lead tetra-acetate failed to yield the products desired. It has now been found that, when *N*-bromosuccinimide, recommended by Kuhn and Münzing[2] for dehydrogenation of simple formazans, is added to 1′ : 5′-diphenyl D-galactoformazan in ethyl acetate, the tetrazolium compound rapidly separates as a colourless oil, which on acetylation gives the same penta-acetate as results from the oxidation of the acetylated formazan.[3]



Experimental.—Dehydrogenation of 1′ : 5′*-diphenyl-*D*-galacto-formazan with* N*-bromosuccinimide.* To the formazan (0·42 g.) in ethyl acetate (50 ml.), *N*-bromosuccinimide (0·5 g.) in ethyl acetate (25 ml.) was added at room temperature. In a few minutes the dark red solution

[1] Zemplén, Mester, and Eckhart, *Chem. Ber.*, 1953, **86**, 472.
[2] Kuhn and Münzing, *ibid.*, p. 858.
[3] Zemplén, Mester, Messmer, and Eckhart, *Acta Chim. Acad. Sci. Hung.*, 1952, **2**, 25.

changed to pale yellow and the tetrazolium compound separated as a pale yellow oil (0·41 g., 81%), $[\alpha]_D^{20}$ +19·1° (in EtOH). To this tetrazolium compound, pyridine (5 ml.) and acetic anhydride (3 ml.) were added. After 24 hr. at room temperature crystals were observed and ethyl acetate (8 ml.) was added. Crystallisation from absolute alcohol gave pale yellow needles (0·3 g.), m. p. 233—234°, $[\alpha]_D^{20}$ +43·5° (in EtOH), not depressed in m. p. on admixture with the acetate described below.

*Dehydrogenation of penta-*O*-acetyl-1′ : 5′-diphenyl-*D*-galactoformazan with* N*-bromosuccinimide.* To the formazan acetate (3·0 g.) in ethyl acetate (50 ml.) an equivalent amount of *N*-bromosuccinimide in ethyl acetate (140 ml.) was added at room temperature. In a few minutes the dark red solution changed to pale yellow. In 10 min. 5-galacto-*penta-acetoxypentyl*-2 : 3-*diphenyltetrazolium bromide* (4·0 g.) separated which, crystallised from absolute ethanol, had m. p. 233—234°, $[\alpha]_D^{20}$ +57·7° (in EtOH) (1·8 g.) (Found : N, 8·4; Ac, 32·0. $C_{28}H_{31}O_{10}N_4Br$ requires N, 8·45; Ac, 32·4%).

We thank Miss Ilona Batta for the microanalyses.

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753. *The Interaction of Iron and Cobalt Nitrosyl Carbonyls with Triaryl Phosphites and Triaryl-phosphines, -arsines, and -stibines.*

By L. MALATESTA and A. ARÀNEO.

IT is known that carbon monoxide in nickel tetracarbonyl can be partially or completely replaced by ligands containing tervalent phosphorus as a donor atom, giving products of the type $Ni(PCl_3)_4$, $Ni(CO)\{P(OEt)_3\}_3$, and $Ni(CO)_2(Ph_3P)_2$ which, in general, are more stable than nickel carbonyl itself.[1,2]

Tricarbonylnitrosylcobalt, $Co(CO)_3(NO)$, and dicarbonyldinitrosyliron, $Fe(CO)_2(NO)_2$, are not only isosteric, but also have a distribution of effective charge very similar to that in nickel carbonyl. This had been deduced by one of us on the basis of considerations of electronegativities of the atoms in these complexes,[3] and has now been confirmed experimentally by the measurement of the electric dipole moments.[4]

The above considerations suggested the possibility that carbon monoxide in the nitrosyl carbonyls could be replaced by tervalent phosphorus donors as happens in nickel tetracarbonyl. As mentioned in a preliminary communication [5] these substitutions occur fairly smoothly. The products are listed in the Table.

Compound	M. p.	Colour	Compound	M. p.	Colour
$Fe(NO)_2(Ph_3P)_2$	194°	Brown	$Co(NO)(CO)\{(PhO)_3P\}_2$	86°	Yellow
$Fe(NO)_2\{(PhO)_3P\}_2$	62—72	Orange	$Co(NO)(CO)_2\{(p\text{-}C_6H_4Me)_3As\}$ ...	*ca.* 125 *	Orange-red
$Fe(NO)_2(CO)(Ph_3As)$	100—110 *	Red			
$Fe(NO)_2(CO)\{(p\text{-}C_6H_4Me)_3As\}$	*ca.* 130 *	Red	$Co(NO)(CO)_2\{(p\text{-}C_6H_4Cl)_3As\}$ ...	*ca.* 135 *	Red
$Fe(NO)_2(CO)(Ph_3Sb)$	105—115 *	Orange-red	$Co(NO)(CO)_2(Ph_3Sb)$	100	Red
			$Co(NO)(CO)_2\{(p\text{-}C_6H_4Me)_2Sb\}$ ...	*ca.* 135 *	Red
$Fe(NO)_2(CO)\{(p\text{-}C_6H_4Me)_3Sb\}$	*ca.* 110 *	Red	$Co(NO)(CO)_2\{(p\text{-}C_6H_4Cl)_3Sb\}$ ...	*ca.* 150 *	Red
$Co(NO)(CO)(Ph_3P)_2$	130	Red			

* Decomp.

We could not obtain cobalt derivatives in which all three molecules of carbon monoxide were replaced; even using an excess of triphenyl phosphite, which with nickel tetracarbonyl gives the compound $Ni(CO)\{(PhO)_3P\}_3$,[2] does not replace more than two carbon monoxide molecules from $Co(CO)_3(NO)$. This could be due to the weaker positive charge on the nitrosyl group bound to a metal, compared with carbon monoxide, which would make

[1] Wilkinson, *J. Amer. Chem. Soc.*, 1951, **73**, 5501.
[2] Malatesta and Sacco, *Ann. Chim.* (*Italy*), 1954, **44**, 134.
[3] Malatesta, *Gazzetta*, 1953, **83**, 964.
[4] Weiss, *Z. anorg. Chem.*, 1956, **277**, 221.
[5] Malatesta and Aràneo, *Rend. Accad. Naz. Lincei*, 1956, VIII, **20**, 365.

further substitution of the carbon monoxide by the phosphorus ligand more difficult. The latter ligand carries an appreciable positive charge in its co-ordination compounds of this type, as shown by the magnitude of their electric dipole moments.

Tertiary aromatic phosphines replace two molecules of carbon monoxide in $Co(CO)_3(NO)$ and $Fe(CO)_2(NO)_2$, as they do in $Ni(CO)_4$, while tertiary aromatic arsines and stibines appear to be able to replace only one molecule of carbon monoxide in all cases.

The colour of the substitution products of tricarbonylnitrosylcobalt with triarylphosphites, -phosphines, -arsines, and -stibines ranges from yellow to brick-red. These products are all stable to air in the crystalline state, and also fairly stable in solution, in which they are monomeric. The solids are diamagnetic. The pure compounds can be obtained either by reprecipitation of a benzene solution with methanol, or by preparing the product in alcohol.

The iron derivatives, which are slightly more coloured than the corresponding cobalt compounds, are almost all less stable than their cobalt and nickel analogues, and are oxidised spontaneously in air when not perfectly dry and stored at low temperatures. They can be recrystallised from organic solvents and are diamagnetic and monomeric in solution.

Experimental.—Before the analyses the complexes were destroyed with concentrated sulphuric acid and potassium sulphate. The iron was determined oxidimetrically with permanganate, phosphorus as $Mg_2P_2O_7$, and nitrogen by Dumas's method on a semimicro-scale.

Molecular weights were determined cryoscopically in 2% benzene solution; the magnetic susceptibilities were measured by the Gouy method.

Dinitrosylbis(triphenylphosphine)iron. A concentrated toluene solution of triphenylphosphine (5·2 g.) was added to finely powdered $Fe(NO)_2(CO)_2$ (1·7 g.) in an inert atmosphere. After 6—8 hr., when gas evolution had ceased, an equal volume of cold absolute methanol was added, and the crystalline *product* (3·5 g.) filtered off and washed with methanol (Found: N, 4·35; P, 9·7; Fe, 8·75%; *M*, 635. $C_{36}H_{30}O_2N_2P_2Fe$ requires N, 4·4; P, 9·7; Fe, 8·8%; *M*, 640); it had χ_M -340×10^{-6}.

Dinitrosylbis(triphenyl phosphite)iron was prepared and purified as above from $Fe(NO)_2(CO)_2$ (1·7 g.) and triphenyl phosphite (6·2 g.) (yield, 2·5 g.) (Found: N, 3·9; P, 8·7; Fe, 7·7. $C_{36}H_{30}O_8N_2P_2Fe$ requires N, 3·8; P, 8·4; Fe, 7·6%). It is less stable than its triphenylphosphine analogue.

Carbonyldinitrosyltriphenylarsineiron. A saturated solution of triphenylarsine (3 g.) in toluene was added to $Fe(NO)_2(CO)_2$ (1·7 g.) under nitrogen. There was lively gas evolution and crystals of product began to separate. After 30 min., before the reaction was complete, the solid was filtered off in a nitrogen atmosphere and washed with cold absolute ethanol (yield, 2 g.). This *compound* had χ_M -215×10^{-6} and decomposed in air within a few days (Found: N, 6·35; As, 16·3; Fe, 12·7. $C_{19}H_{15}O_3N_2AsFe$ requires N, 6·2; As, 16·7; Fe, 12·4%).

The following compounds were prepared and purified as the arsine derivative from stoicheiometric amounts of reactants (1·7 g. of nitrosylcarbonyl):

*Carbonyldinitrosyltri-*p*-tolylarsineiron,* stable in air for a few days (yield, 2 g.) (Found: N, 5·6; As, 15·0; Fe, 11·6. $C_{22}H_{21}O_3N_2AsFe$ requires N, 5·7; As, 15·2; Fe, 11·4%).

Carbonyldinitrosyltriphenylstibineiron, stable in air only for a few hours (yield, 1·2 g.) (Found: N, 5·7; Sb, 24·4; Fe, 11·3. $C_{19}H_{15}O_3N_2FeSb$ requires N, 5·6; Sb, 24·4; Fe, 11·2%).

*Carbonyldinitrosyltri-*p*-tolylstibineiron* (yield, 2 g.) (Found: N, 5·3; Sb, 22·5; Fe, 10·5. $C_{22}H_{21}O_3N_2FeSb$ requires N, 5·2; Sb, 22·6; Fe, 10·4%).

Carbonylnitrosylbis(triphenylphosphine)cobalt, prepared and purified analogously to the corresponding iron compound from $Co(CO)_3(NO)$ (1·7 g.) and triphenylphosphine (5·2 g.) (yield, 3·5 g.) (Found: N, 2·2; P, 10·2; Co, 9·1%; *M*, 690. $C_{37}H_{30}O_2NP_2Co$ requires N, 2·2; P, 9·9; Co, 9·2%; *M*, 641).

Similarly prepared [from $Co(CO)_3(NO)$ (1·7 g.) and the stoicheiometric amount of ligand] were:

Carbonylnitrosylbis(triphenyl phosphite)cobalt (yield, 5 g.) (Found: N, 2·0; P, 8·6; Co, 8·0. $C_{37}H_{30}O_8NP_2Co$ requires N, 1·9; P, 8·4; Co, 8·0%), χ_M -420×10^{-6}.

Dicarbonylnitrosyltri-p-*tolylarsinecobalt* (yield, 4 g.) (Found: N, 2·9; As, 14·9; Co, 11·6. $C_{23}H_{21}O_3NAsCo$ requires N, 2·8; As, 15·2; Co, 12·0%).

Dicarbonylnitrosyltri-p-*chlorophenylarsinecobalt* (yield, 3·5 g.) (Found: N, 2·6; As, 13·3; Co, 10·0. $C_{20}H_{12}O_3NCl_3AsCo$ requires N, 2·5; As, 13·5; Co, 10·4%).

Dicarbonylnitrosyltriphenylstibinecobalt (yield, 3·5 g.) (Found: N, 2·9; Sb, 24·5; Co, 11·6. $C_{20}H_{15}O_3NCoSb$ requires N, 2·8; Sb, 24·5; Co, 11·85%).

Dicarbonylnitrosyltri-p-*tolylstibinecobalt* (yield, 3 g.) (Found: N, 2·6; Sb, 22·8; Co, 10·7%; *M*, 544. $C_{23}H_{21}O_3NCoSb$ requires N, 2·6; Sb, 22·5; Co, 10·9%; *M*, 540).

Dicarbonylnitrosyltri-p-*chlorophenylstibinecobalt* (yield, 4 g.) (Found: N, 2·4; Sb, 20·6; Co, 9·4. $C_{20}H_{12}O_3NCl_3CoSb$ requires N, 2·3; Sb, 20·2; Co, 9·7%).

The present work was supported by a grant from the Italian Research Council.

THE UNIVERSITY, MILAN. [*Received, March 25th*, 1957.]

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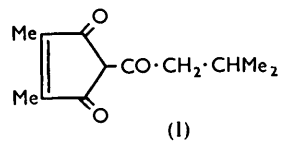
754. β-*Triketones. Part IV.* The Chromophore of Calythrone.*

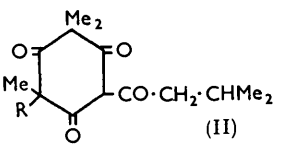
By A. J. BIRCH and R. J. ENGLISH.

THE formula (I) was assigned to calythrone [1] chiefly on the basis of published work,[2] and the formula was considered [3] to be supported by an examination of spectra. However, no models containing the chromophore of structure (I) appear to have been made and we have accordingly synthesised one for comparison.

Attempts to condense dimethylmaleic anhydride or ester with ketones in the presence of sodium ethoxide, sodium amide, or boron trifluoride failed to produce the desired triketones.[4] Another possible route is based on the suggested biosynthesis [3] of calythrone. This would involve the conversion of the *cyclo*hexanetrione (II; R = H) into the triketo-alcohol (II; R = OH) and then by the action of alkali into the *cyclo*pentane derivative (III; R = R′ = Me, R″ = Bu^i), followed by dehydration to calythrone (I). However, 3 : 3 : 5-trimethylphloracetophenone was not oxidised with oxygen and lead acetate as in the reaction employed in the analogous humulone series.[5] This type of reaction is known to fail or to give very poor yields in some cases.



(I)



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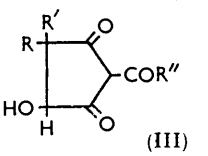
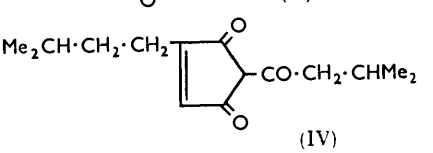


(III)



(IV)

At this stage, through the kindness of Dr. S. David (Nancy), we obtained access to dihydrohumulinic acid (III; R = H, R′ = CH_2Bu^i, R″ = Bu^i), dehydration of which with polyphosphoric acid gave the compound (IV), whose ultraviolet absorption [$\lambda_{max.}$ 240, 266 mμ (log ε 4·22, 4·26)] is very similar to that [3] of calythrone [$\lambda_{max.}$ 240, 266 mμ (log ε 4·29, 4·26)]. The infrared spectrum also is similar to that of calythrone in the region 1700—1600 cm.$^{-1}$ (in CS_2). There is a sharp peak at 1710 cm.$^{-1}$, presumably due to an

* Part III, Birch and Elliott, *Austral. J. Chem.*, 1956, **9**, 238.

[1] Birch, *J.*, 1951, 3026.
[2] Cahn, Gibson, Penfold, and Simonsen, *J.*, 1931, 286.
[3] Birch and Elliott, *Austral. J. Chem.*, 1956, **9**, 95.
[4] Birch and Murray, unpublished work.
[5] Campbell and Coppinger, *J. Amer. Chem. Soc.*, 1951, **73**, 1849.

αβ-unsaturated ketone grouping in a five-membered ring; this band is observable in the spectrum of dihydrohumulinic acid. A broad peak at 1620 cm.$^{-1}$ extending below 1500 cm.$^{-1}$ is present in both and appears to be characteristic of β-triketones of this type. Dihydrohumulinic acid has two bands in the hydroxyl region, 3560 and 3450 cm.$^{-1}$, the latter of which is missing from the spectrum of (IV).

It is notable that dihydrohumulinic acid is soluble in bicarbonate solutions, whereas the triketone (IV) is soluble only in carbonate solutions, to give a yellow salt.

Experimental.—*4-3'-Methylbutyl-2-isovaleroylcyclopent-4-ene-1 : 3-dione.*—Dihydrohumulinic acid (100 mg.) in a mixture of phosphoric acid (*d* 1·75; 4 c.c.) and phosphoric oxide (4 g.) was heated at 130° for 8 min. The *product* obtained by dilution with water, ether-extraction, and crystallisation from ether–pentane, had m. p. 75—76° (40 mg.) (Found: C, 72·0; H, 8·7. $C_{15}H_{22}O_3$ requires C, 72·0; H, 8·8%). It gave a deep brownish-red colour with alcoholic ferric chloride. Refluxing with powdered cupric acetate in methanol, dilution with water, and ether-extraction gave the copper salt as a greenish-yellow solid, m. p. 145—147° (from benzene).

We are indebted to the Cumberland Educational Committee for a grant (to R. J. E.).

THE UNIVERSITY OF MANCHESTER. [*Received, April 14th,* 1957.]

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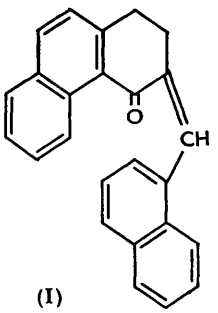
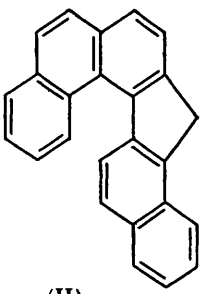
755. *A Synthesis of 7 : 8-Benzonaphtho(2' : 1'-3 : 4)fluorene.*

By NG. PH. BUU-HOÏ and G. SAINT-RUF.

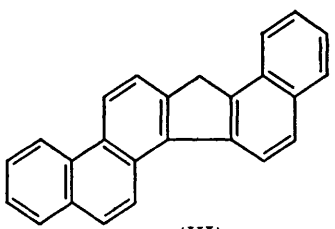
SEVERAL years ago, Buu-Hoï and Cagniant showed that the application of the Rapson–Shuttleworth cyclodehydration reaction [1] to 2-naphthylidene derivatives of 1-tetralone and more complex ketones afforded a convenient route to several condensed polycyclic fluorenes.[2] In those studies, the cyclodehydration of 1 : 2 : 3 : 4-tetrahydro-3-1'-naphthylmethylene-4-oxophenanthrene (I) was investigated and a hydrocarbon, m. p. 285°, isolated and assigned the tentative structure of 7 : 8-benzonaphtho(2' : 1'-3 : 4)fluorene (II). This hydrocarbon, unknown at that time, was later prepared by Campbell from dimerisation products of β-2-naphthylpropiolic acid,[3] and found to melt at 138—139°. This led us to re-investigate the constitution of the hydrocarbon, m. p. 285°, which now proved to be the isomeric 7 : 8-benzonaphtho(1' : 2'-3 : 4)fluorene (III), previously prepared by



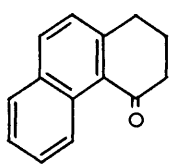
(I)



(II)



(III)



(IV)

Buu-Hoï and Cagniant.[4] This result could be explained by assuming either that a rearrangement occurred during the cyclodehydration of (I), as in the synthesis of 1 : 2 : 5 : 6-dibenzofluorene,[4] or that the starting ketone (IV) contained some of the isomeric 1-ketone if the recorded method [5] for the separation of β-1- and β-2-naphthoylpropionic acid is insufficient. The latter was found to be the case, as the pure 4-ketone obtained from

[1] Rapson and Shuttleworth, *J.*, 1940, 636.
[2] Buu-Hoï and Cagniant, *Rev. Sci.*, 1942, **80**, 319.
[3] Campbell, *J.*, 1954, 3659.
[4] Buu-Hoï and Cagniant, *Rev. Sci.*, 1942, **80**, 384; 1943, **81**, 30.
[5] Haworth, *J.*, 1932, 1125.

γ-2-naphthylbutyric acid which had been prepared by a malonic synthesis *via* pure 2-β-naphthylethanol [6] gave, with 1-naphthaldehyde, the condensation product of (I) with a melting point substantially higher than that previously recorded. Cyclodehydration of this product afforded, in good yield, 7 : 8-benzonaphtho(2′ : 1′-3 : 4)fluorene (m. p. 147°, higher than that recorded by Campbell, but giving the same absorption spectrum and the same dipicrate). This observation offers a convenient route to this difficultly accessible hydrocarbon (II), whose carcinogenic activity is being determined. Cyclodehydration of a sample of compound (I) prepared from the 4-ketone (IV) *via* a sample of γ-2-naphthylbutyric acid obtained according to the literature,[5] gave small amounts of hydrocarbon (III), together with the isomeric hydrocarbon (II).

Experimental.—1 : 2 : 3 : 4 - *Tetrahydro* - 3 - 1′ - *naphthylmethylene* - 4 - *oxophenanthrene* (I). 1 : 2 : 3 : 4-Tetrahydro-4-oxophenanthrene (7 g.; m. p. 69°), prepared by cyclisation of pure α-2-naphthylbutyric acid, m. p. 99° (lit.,[5] m. p. 95—96°), with sulphuric acid, was shaken with α-naphthaldehyde (5·6 g.) in ethanol (50 c.c.) and a few drops of 30% concentrated aqueous potassium hydroxide and left overnight at room temperature. The solid precipitate formed was recrystallised twice from ethanol, giving pale yellow prisms (6 g.), m. p. 173° (Found: C, 89·7; H, 5·4. Calc. for $C_{25}H_{18}O$: C, 89·8; H, 5·4%). The product similarly prepared from impure ketone melted at 152—153° with sintering, and could not be purified further.

Cyclodehydration. A solution of the ketone (I) (5·5 g.; m. p. 173°) in anhydrous xylene (100 c.c.) was refluxed with finely powdered phosphoric oxide (4 g.) for 30 hr. After cooling, the dark violet mixture was poured into water (500 c.c.), the xylene solution washed with dilute aqueous sodium hydroxide, then with water, and dried (Na_2SO_4), the solvent removed and the residue distilled *in vacuo*. The portion, b. p. 285—290°/0·4 mm., which did not solidify, was taken up in benzene and converted into a dipicrate, which formed brown-red needles, m. p. 175°, from benzene (lit.,[3] m. p. 175—176°). Decomposition of this with aqueous ammonia yielded 7 : 8-benzonaphtho(2′ : 1′-3 : 4)fluorene (II), leaflets (1 g.), m. p. 147° (from ethanol–benzene) (Found: C, 94·7; H, 5·2. Calc. for $C_{25}H_{16}$: C, 94·9: H, 5·1%); Campbell [3] gave m. p. 138—139°.

Cyclodehydration of a sample of compound (I) prepared from impure material yielded, after the usual treatment, a resinous distillate which was triturated with *cyclo*hexane; the small amount of solid obtained (0·1 g.) was recrystallised from benzene, giving needles, m. p. 286—287°, of 7 : 8-benzonaphtho(1′ : 2′-3 : 4)fluorene (III). The portion dissolved in *cyclo*hexane was freed from solvent and treated with a benzene solution of picric acid, giving the dipicrate (1·2 g.) of hydrocarbon (II). Both the condensed fluorenes gave intense violet fluorescence in benzene solution.

THE RADIUM INSTITUTE, THE UNIVERSITY OF PARIS. [*Received, April 15th*, 1957.]

[6] Karrer, Geiger, Ruegger, and Schwab, *Helv. Chim. Acta*, 1940, **23**, 585; Sontag, *Ann. Chim.*, 1934, **1**, 359.

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756. *Some Aromatic Aldehydes.*

By KENNETH CLARKE.

SEVERAL disubstituted benzaldehydes were required as intermediates in another investigation. Preparation of di-*ortho*-substituted aldehydes proved extremely difficult, especially if one or both of the substituents were electron-attracting. Oxidation of the corresponding toluenes failed completely or gave very poor yields. The Sommelet reaction fails [1,2] with both di-*ortho*-substituted benzyl halides and also with benzyl halides substituted by more than one electron-attracting group.[3] Hass and Bender's reaction,[4] whereby aldehydes are obtained from benzyl halides after condensation with nitropropane, also fails for nitrobenzaldehydes.[3]

[1] Angyal, Morris, Rassack, and Waterer, *J.*, 1949, 2704.
[2] Fuson and Denton, *J. Amer. Chem. Soc.*, 1941, **63**, 654.
[3] Angyal, Morris, Tetaz, and Wilson, *J.*, 1950, 2141.
[4] Hass and Bender, *J. Amer. Chem. Soc.*, 1949, **69**, 1767.

Kröhnke's method,[5] however, proved more satisfactory. This involves the preparation, from the requisite benzyl bromide, of the quaternary 1-benzylpyridinium salt, which with *NN*-dimethyl-*p*-nitrosoaniline will give a nitrone that can be split by mineral acid, thus forming the required aldehyde.

$$Ph{\cdot}CH_2Br \longrightarrow PhCH_2{\cdot}\overset{+}{N}C_5H_5\ Br^- \longrightarrow Ph{\cdot}CH{:}N(O){\cdot}C_6H_4{\cdot}NMe_2 \longrightarrow Ph{\cdot}CHO$$

By this method 2 : 6-dinitro- (58%), 2 : 6-dichloro- (50%), 2-nitro-4- (57%), and -6-chloro- (75%), and 2-bromo-6-nitro-benzaldehydes (79%) have been prepared in the overall yields given in parentheses. The three monofluoro-benzaldehydes were obtained in yields of only 15—20%; consequently the method has no advantage over those already available for these compounds.

Experimental.—1-(2-*Bromo*-6-*nitrobenzyl*)*pyridinium bromide.* Bromine (3 ml.) was added during 1 hr. to 2-bromo-6-nitrotoluene at 170°; the mixture was then illuminated (2 × 200 w) and the temperature maintained at 170° until the bromine had disappeared (2—3 hr.). Pyridine (7 ml.) and ethanol (10—15 ml.) were added to the cooled mixture, which was then heated for 1 hr. on a steam-bath. Cooling and recrystallisation (ethanol) gave the salt (16 g.) as prisms, m. p. 210°.

2-*Bromo*-6-*nitrophenyl*-N-p-*dimethylaminophenylnitrone.* N-Sodium hydroxide (60 ml.) was gradually added at 0° to a stirred suspension of 1-(2-bromo-6-nitrobenzyl)pyridinium bromide (8·0 g.) and *NN*-dimethyl-*p*-nitrosoaniline (4·0 g.) in ethanol (100 ml.), stirring was then continued for 2—3 hr., and the solution finally diluted. The precipitated nitrone was washed and crystallised (ethyl acetate), giving red-brown prisms (6·5 g.), m. p. 162°.

Compound R	R′	M. p.	Solv.	Formula	Found, % C	H	N	Reqd., % C	H	N
Benzylpyridinium bromides, $RR'C_6H_3{\cdot}CH_2{\cdot}N^+C_5H_5\ Br^-$										
2 : 6-Cl_2		220°	EtOH	$C_{12}H_9NCl_2Br$	45·5	3·15	4·1	45·2	3·1	4·4
2 : 6-$(NO_2)_2$ [a]		166	,,	$C_{12}H_{10}O_4N_3Br$	41·8	3·3	12·3	42·4	2·9	12·4
2-Cl : 6-NO_2		192	,,	$C_{12}H_9O_2N_2ClBr$	43·9	3·3	8·2	43·7	3·0	8·5
4-Cl : 2-NO_2		203	,,	,,	43·9	3·2	8·4	,,	,,	,,
2-Br : 6-NO_2		210	,,	$C_{12}H_9O_2N_2Br_2$	38·6	2·6	7·4	38·6	2·4	7·5
2-F, H [b]		136	$COMe_2$	$C_{12}H_{11}NBrF$	—	—	—	—	—	—
3-F, H		148	,,	,,	53·6	4·1	—	53·7	4·1	—
4-F, H [c]		57—58	$COMe_2$ +EtOH	,,	—	—	—	—	—	—
Nitrones, $RR'C_6H_3{\cdot}CH{:}N(O){\cdot}C_6H_4Me_2$										
2 : 6-Cl_2 [d]		156	EtAc	$C_{15}H_{14}ON_2Cl_2$	58·0	4·5	—	58·3	4·5	—
2 : 6-$(NO_2)_2$		184	,,	$C_{15}H_{14}O_5N_4$	54·2	4·1	16·7	54·3	4·2	17·0
2-Cl : 6-NO_2		165	,,	$C_{15}H_{14}O_3N_3Cl$	56·1	4·6	13·1	56·3	4·4	13·1
4-Cl : 2-NO_2		166	,,	,,	56·6	4·6	—	,,	,,	,,
2-Br : 6-NO_2		162	,,	$C_{15}H_{14}O_3N_3Br$	49·6	3·8	—	49·5	3·9	—
2-F, H		83	EtOH	$C_{15}H_{15}ON_2F$	—	—	10·8	—	—	10·9
3-F, H		100	,,	,,	—	—	10·9	—	—	,,
4-F, H		127	,,	,,	—	—	10·7	—	—	,,

[a] *Perchlorate,* m. p. 174° (Found: C, 40·4; H, 2·8; N, 11·4. $C_{12}H_{10}O_8N_3Cl$ requires C, 40·1; H, 2·8; N, 11·7%). [b] *Perchlorate,* m. p. 100° (Found: C, 49·9; H, 4·1. $C_{12}H_{11}O_4NClF$ requires C, 50·1; H, 3·8%). [c] *Perchlorate,* m. p. 96° (Found: C, 49·8; H, 4·1%).

[d] The method described above gave only a low yield. Yields were, however, satisfactory when the reaction mixture was kept overnight with sodium ethoxide.

2-Bromo-6-nitrobenzaldehyde. The nitrone (10 g.) was heated for 5 min. with 6N-sulphuric acid (150 ml.) on a steam-bath. When recrystallised (from ethanol) the aldehyde formed pale yellow prisms (6·1 g.), m. p. 82°.

Compounds detailed in the Table were produced similarly.

The author thanks Imperial Chemical Industries Limited for a grant.

THE UNIVERSITY, HULL. [*Received, 16th April,* 1957.]

[5] Kröhnke and Borner, *Ber.*, 1936, **69**, 2006; 1938, **71**, 2583.