# NOTES.

## 60. *Benzylsodium and Cumylpotassium as Initiators of Polymerization.*

By R. ASAMI, M. LEVY, and M. SZWARC.

STUDIES of the transformation which slowly takes place in the solution of " living " polystyrene [1] prompted us to investigate benzylsodium and cumylpotassium which may be considered as models of " living " polystyrene and " living " poly-α-methylstyrene.

Ultraviolet spectra.

| Species | $\lambda_{max.}$ (mμ) | $10^4\varepsilon$ | Species | $\lambda_{max.}$ (mμ) | $10^4\varepsilon$ |
|---|---|---|---|---|---|
| Benzylsodium ..... | 355 | 1·2 | " Living " polystyrene ($Na^+$) ..... | 340 | 1·4 |
| Cumylpotassium ..... | 338 | 1·8 | " Living " poly-α-methylstyrene ($Na^+$) | 350 | 1·4 |

Benzylsodium was prepared by reaction of dibenzylmercury in tetrahydrofuran with a sodium mirror,[2] this method being more satisfactory than reaction of phenylsodium with toluene.[3] Cumylpotassium was prepared by the Ziegler method [4] from the appropriate ether.

[1] Spach, Levy, and Szwarc, *J.*, 1961, 355.
[2] Schlenk and Holtz, *Ber.*, 1917, **50**, 269.
[3] Gilman, Pacevitz, and Baine, *J. Amer. Chem. Soc.*, 1940, **62**, 1514.
[4] Ziegler and Dislich, *Ber.*, 1957, **90**, 1107.

All the preparations and the subsequent manipulations were performed on a high-vacuum line with all-glass equipment, using break-seals and constrictions instead of stopcocks.

*Spectra.* Spectra of benzylsodium and cumylpotassium in tetrahydrofuran showed a comparatively narrow band, with the peak at 355 mμ for the former (see Fig. 1) and at 338 mμ for the latter, resembling those of " living " polystyrene and " living " poly-α-methylstyrene (cf. Table). There is thus no doubt that the " living " polymers possess the ~CHPh$^-$Na$^+$ and ~CPhMe$^-$Na$^+$ end group, respectively.

On storage at room temperature for a week no change was observed in the spectrum of cumylpotassium, but for benzylsodium a new peak appeared at 485 mμ (see Fig. 1)

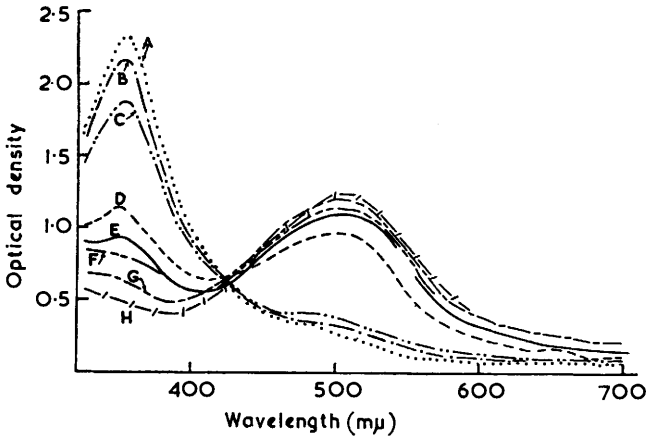


FIG. 1. Spectra of benzylsodium in tetrahydrofuran on storage for (A) 3, (B) 7, (C) 10, (D) 45, (E) 72, (F) 97, (G) 123, and (H) 169 hr.

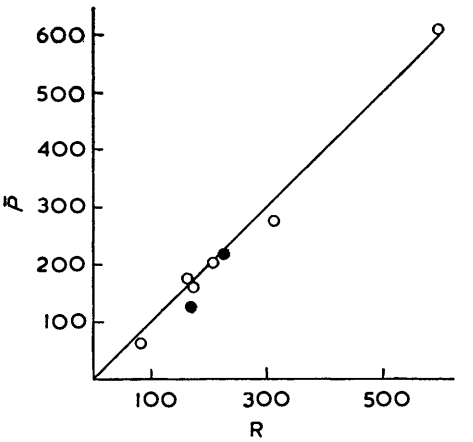


FIG. 2. Correlation of [styrene] : [initiator] ratio (R) with the degree of polymerisation ($\bar{P}$) of the resulting polystyrene. ($\bar{P} = 1{\cdot}7 \times 10^5[\eta]^{1{\cdot}35}/104$). ● Benzylsodium. ○ Cumylpotassium.

within a few hours and the original peak began to decline. Although this phenomenon superficially resembles that observed in the " living " polystyrene system,[1] it must be due to a different sequence of reactions since the coloured species absorbs at shorter wavelength (485 mμ) than does the new ion formed from " living " polystyrene ($\lambda_{max.}$ 535 mμ). That cumylpotassium appeared to be stable indicates again that the α-hydrogen atom is involved in the process. No attempt was made to elucidate the reaction or to identify the new species. Probably dimeric or polymeric species, formed by the ion-pair association, are involved which eventually produces, by some unknown mechanism, derivatives of polybenzyl. We found also that in this reaction a substantial fraction of the original organometallic compound produced colourless species.

The original benzylsodium formed phenylacetic acid on carboxylation (76% yield), but the transformed product produced a negligible amount of this acid.

Spectra of benzylsodium in tetrahydrofuran were studied by Kuwata.[5] His absorption curve closely resembled that obtained in our laboratory for the transformed benzylsodium. It appears from Kuwata's report that his solution had been in contact with metallic sodium for a long time and our findings demonstrate that this is sufficient to destroy the original compound.

*Initiation of Polymerization.*—Addition of styrene to the solution of benzylsodium or cumylpotassium initiates polymerization. The intrinsic viscosity of the products were determined in an Ubbelohde viscometer and compared with the ratio monomer : initiator. The results are shown graphically in Fig. 2. The agreement between the degree of polymerization ($\bar{P}_n$) obtained from the viscosity and from the ratio proves that the rate constant of initiation is comparable with that of propagation. On the other hand, the polymerization initiated by the transformed benzylsodium produced a polymer of $[\eta]$ 0·923 (in units of 100 c.c./g.), corresponding to $\bar{P}_n = 1470$, while the ratio monomer : initiator = 169. Obviously, the product formed from benzylsodium on prolonged storage is a poor initiator, while cumylpotassium and freshly prepared benzylsodium are efficient initiators for "living" polymers.

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[5] Kuwata, *Bull. Chem. Soc. Japan*, 1960, **33**, 1091.

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## 61. *Decomposition of Ferrocinium Salts in Organic Solvents.*

By R. M. Golding and L. E. Orgel.

Theoretical arguments suggest that the ground state of the ferrocinium ion, $[Fe(C_5H_5)_2]^+$, is orbitally degenerate,[1] therefore it should not show electron resonance at room temperature. We have obtained no resonance from powdered ferrocinium bromide at room or at liquid-nitrogen temperature. The observation of spin-resonance from solutions of ferrocinium ion in acetone reported by Nöth *et al.*[2] seemed thus of particular interest and led us to investigate further this and related systems.

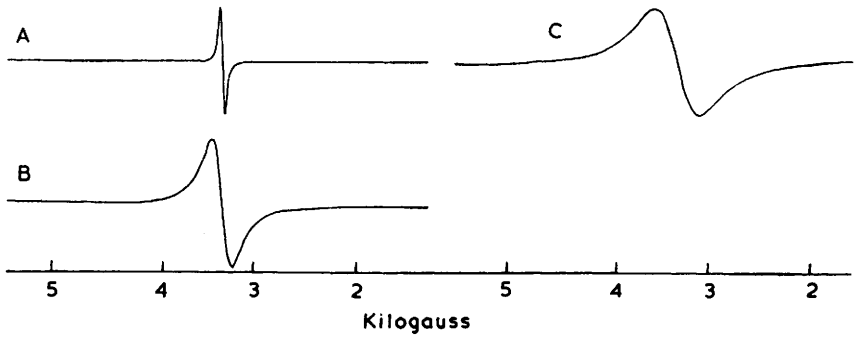
We first noticed that the blue ferrocinium bromide dissolves in dry acetone to give a red-brown solution and that the addition of alkali-metal bromides to the green solution of

Electron spin resonance of ferric chloride, bromide, and thiocyanate in dry acetone.

| Ferric salt | Br | CNS | Cl |
|---|---|---|---|
| *g* | 2·052 | 2·011 | 2·016 |
| Distance between peaks of the derivative curve (gauss) | 550 | 260 | 55 |

ferrocinium picrate in acetone gave the same red-brown solution. The red-brown, bromide-containing solutions show paramagnetic resonance absorption but the green picrate solution does not. At first we believed that we were studying a complex of the $[Fe(C_5H_5)_2]^+$ ion with the bromide ion, analogous to the thiocyanate studied by Smith,[3] but later found the same resonance for ferric bromide in acetone. In the presence of chloride or thiocyanate, ferrocinium solutions in acetone decompose to give the corresponding ferric salts. Similar reactions occur in tetrahydrofuran solutions. It should be noted that our results do not invalidate Smith's conclusions concerning ferrocinium thiocyanate since we used much more concentrated solutions.

[1] Robertson and McConnel, *J. Phys. Chem.*, 1960, **64**, 70; Levy and Orgel, *Mol. Phys.*, 1961, **4**, 93.
[2] Nöth, Voitlander, and Nussbaum, *Naturwiss.*, 1960, **47**, 57.
[3] Smith, *J.*, 1961, 473.




Electron resonance signals of solutions of ferric salts in dry acetone: A, chloride; B, thiocyanate; C, bromide.

The electron resonance signals of solutions of ferric chloride, bromide, and thiocyanate in dry acetone are shown in the Figure. They all have *g*-values close to 2; the band widths (peak to peak of the derivative curves) are, however, different and reproducible (Table 1).

Attempts to discover the nature of the absorbing species were only partially successful. It is well known that solutions of ferric chloride in polar organic solvents contain a variety of complex ions $[FeCl_n(solvent)_m]^{(3-n)+}$ where $n \leqslant 4$, together with binuclear complexes in certain cases.[4] The reproducibility of the electron-resonance signals under a wide variety of conditions, and particularly the constant value of the peak width makes it very probable that a single species is responsible for all the absorption. The following experimental and theoretical observations suggest, but by no means establish, that in chloride-containing solutions $[FeCl_4]^-$ is the species which shows resonance: (*a*) Addition of lithium chloride to a solution of ferric chloride in acetone at first resulted in an increased absorption. The intensity of absorption became constant at double its value when about 4·5 moles of $Cl^-$ were present for each mole of $Fe^{3+}$. (*b*) Solutions of ferric chloride, etc., in benzene, which presumably contain only $(FeCl_3)_n$ species, show no resonance. (*c*) The $^6A_1$ ground state of the $Fe^{3+}$ ion is split only slightly in a fully cubic environment and the resonance spectrum is expected to be isotropic. Any other ion $[(FeCl_n(solvent)_m]^{(3-n)+}$ (except $[FeCl_6]^{3-}$ which has never been observed in ferric chloride solutions) would have a much larger zero-field splitting and probably an anisotropic *g* value.[5] The observed spectrum could well arise from a $Fe^{3+}$ ion in a cubic environment, but the possibility that it has some other origin cannot be excluded.

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[4] Gamlen and Jordan, *J.*, 1953, 1435; Friedman, *J. Amer. Chem. Soc.*, 1952, **74**, 5; Brealey and Uri, *J. Chem. Phys.*, 1953, **20**, 257.
[5] Griffith, "The Theory of Transition-Metal Ions," Cambridge University Press, 1961, Chapter 12, section 4.5.

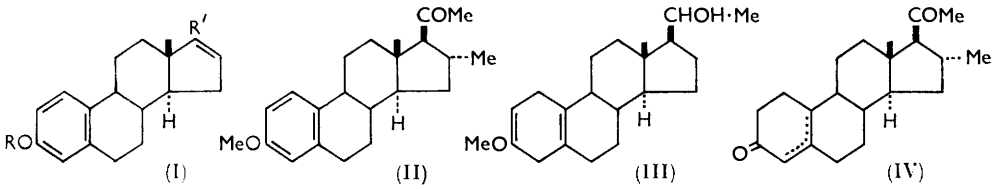
## 62. 16α-*Methyl*-19-*norpregn*-4-*ene*- *and* -5(10)-*ene*-3,20-*diones*.

By D. Burn and V. Petrow.

The compounds named in the title were required for study as claudogens.[1] Œstrone 3-methyl ether with potassium cyanide in acetic acid afforded the cyanohydrin which, without purification, was dehydrated with phosphorus oxychloride in pyridine to the

[1] Petrow, *J. Pharm. Pharmacol.*, 1960, **12**, 1704.

unsaturated nitrile (I; R = Me, R′ = CN). When œstrone 3-acetate was employed [2] a lower overall yield of the acetoxy-cyanide (I; R = Ac, R′ = CN) was obtained. The




(I) (II) (III) (IV)

nitrile and methylmagnesium iodide gave the known ketone [3] (I; R = Me, R′ = Ac), which passed smoothly into 3-methoxy-16α-methyl-19-norpregna-1,3,5(10)-trien-20-one (II) on further treatment with the Grignard reagent. Birch reduction then gave the 1,4-dihydro-derivative (III), careful hydrolysis of which with oxalic acid afforded the 5(10)-unsaturated 3-ketone; use of hydrochloric acid gave the 4-unsaturated ketone. Their oxidation with chromic acid in sulphuric acid and acetone [4] afforded the compounds named in the title, without concomitant bond migration in the case of the 5(10)-unsaturated isomer.[5]

*Experimental.*—Ultraviolet absorption spectra were measured for EtOH solutions.

17-*Cyano*-3-*methoxyœstra*-1,3,5(10),16-*tetraene* (I; R = Me, R′ = CN).—Œstrone methyl ether (9 g.), potassium cyanide (65 g.), ethanol (350 ml.), and acetic acid (65 ml.) were kept at 50° for 6 hr. and then overnight at room temperature. The cyanohydrin was precipitated with water, collected, and dried. It was refluxed in pyridine (90 ml.) with phosphorus oxychloride (22·5 ml.) for ½ hr. and the mixture poured into water. The solid product was collected, dried, and chromatographed on alumina (250 g.) in 4 : 1 benzene–light petroleum (b. p. 40—60°). The benzene–light petroleum eluates were combined and crystallised from dichloromethane–methanol to give 17-*cyano*-3-*methoxyœstra*-1,3,5(10),16-*tetraene* as flakes, m. p. 168—170°, $[\alpha]_D^{20}$ +70° (*c* 0·8 in $CHCl_3$), $\lambda_{max.}$ 217 (ε 16,770), 277 (ε 2550), and 287 mμ (ε 2368) (Found: C, 82·05; H, 7·75; N, 4·95. $C_{20}H_{23}NO$ requires C, 81·85; H, 7·9; N, 4·75%).

3-*Methoxy*-19-*norpregna*-1,3,5(10),16-*tetraen*-20-*one* (I; R = Me, R′ = Ac).—The foregoing nitrile (2·5 g.) in dry benzene (90 ml.) was added to a solution of methylmagnesium iodide [from magnesium (1 g.) and methyl iodide (3 ml.)] in dry ether (50 ml.). The mixture was heated under reflux for 3 hr. and then poured slowly into acetic acid (150 ml.) and water (50 ml.) and steam-distilled. The residue crystallised from dichloromethane–methanol to give the unsaturated ketone (I; R = Me, R′ = COMe) as rods, m. p. 192—194°, $[\alpha]_D^{22}$ +116° (*c* 1·0 in $CHCl_3$) (lit.,[3] m. p. 193—194°, $[\alpha]_D$ +115°).

3-*Methoxy*-16α-*methyl*-19-*norpregna*-1,3,5(10)-*trien*-20-*one* (II).—The foregoing unsaturated ketone (3·1 g.) in dry tetrahydrofuran (75 ml.) was added under nitrogen to methylmagnesium iodide [from 0·6 g. of magnesium in ether (25 ml.) and tetrahydrofuran (50 ml.) with subsequent removal of the ether] containing cuprous bromide (0·1 g.), and the mixture was stirred at room temperature for 2 hr. Aqueous ammonium chloride was added and the product was isolated with ether. Evaporation of the extract left a gum which crystallised from ethanol to give 3-*methoxy*-16α-*methyl*-19-*norpregna*-1,3,5(10)-*trien*-20-*one* as laths, m. p. 116—118°, $[\alpha]_D^{25}$ +143° (*c* 0·65 in $CHCl_3$), $\lambda_{max.}$ 278 (ε 2080) and 287 mμ (ε 1990) (Found: C, 81·15; H, 8·95. $C_{22}H_{30}O_2$ requires C, 80·95; H, 9·25%).

16α-*Methyl*-19-*norpregn*-4-*ene*-3,20-*dione* (IV; Δ[4]).—A solution of the ketone (II) (3 g.) in dry ether (150 ml.) was added slowly to one of lithium (1·5 g.) in liquid ammonia (500 ml.), and the mixture stirred under reflux for 1 hr. Methanol (50 ml.) was added slowly, after which the ammonia was allowed to evaporate overnight. The product, isolated with ether, was heated under reflux in methanol (75 ml.) and 2N-hydrochloric acid (25 ml.) for 1 hr. and again isolated with ether. It was oxidised in acetone (70 ml.) with Jones's reagent [4] (2 ml.). The solid obtained on pouring the mixture into water was chromatographed on alumina (100 g.) in 1 : 1 benzene–light petroleum (b. p. 40—60°). The solids eluted with benzene were combined

[2] Velluz and Muller, *Bull. Soc. chim. France*, 1950, 166.
[3] Sondheimer, Neumann, Ringold, and Rosenkranz, *J. Amer. Chem. Soc.*, 1954, **76**, 2230.
[4] Bowden, Heilbron, Jones, and Weedon, *J.*, 1946, 39.
[5] Djerassi, Engle, and Bowers, *J. Org. Chem.*, 1956, **21**, 1547.

and crystallised from aqueous acetone to give 16α-*methyl*-19-*norpregn*-4-*ene*-3,20-*dione* as laths, m. p. 135—137°, $[\alpha]_D^{24}$ +143° (*c* 1·15 in $CHCl_3$), $\lambda_{max.}$ 239 mμ (ε 15,470) (Found: C, 80·4; H, 9·7. $C_{21}H_{30}O_2$ requires C, 80·2; H, 9·6%).

16α-*Methyl*-19-*norpregn*-5(10)-*ene*-3,20-*dione* (IV; $\Delta^{5(10)}$).—The ketone (II) (3·2 g.) was reduced with lithium in liquid ammonia as in the previous example, and the product kept for 2 hr. at room temperature in methanol (200 ml.) and water (15 ml.) containing oxalic acid dihydrate (2·5 g.). After neutralisation with sodium carbonate the product was isolated with ether and oxidised, under nitrogen, with Jones's reagent (2 ml.) as before. The solid obtained on pouring the mixture into water was chromatographed on *neutral* alumina in 1 : 1 benzene–light petroleum (b. p. 40—60°), the eluted gums showing only saturated carbonyl absorption in the infrared region. Crystallisation from aqueous acetone afforded 16α-*methyl*-19-*norpregn*-5(10)-*ene*-3,20-*dione* as flakes, m. p. 121—123°, $[\alpha]_D^{24}$ +199·5° (*c* 0·5 in $CHCl_3$), $\lambda_{max.}$ 287 mμ (ε 81) (Found: C, 80·0; H, 9·4. $C_{21}H_{30}O_2$ requires C, 80·2; H, 9·6%).

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## 63. *Formation of Stereospecific* $DO_2C{\cdot}CHD{\cdot}C\dot{H}{\cdot}CO_2D$ *on Irradiation of* OO′-*Dideuteriosuccinic Acid.*

By D. POOLEY and D. H. WHIFFEN.

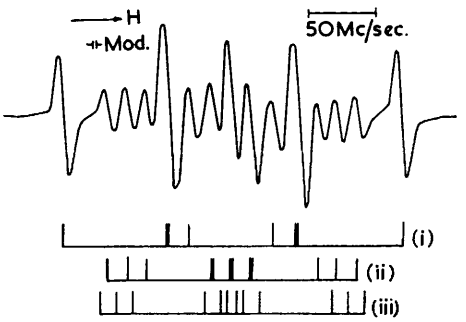
IN connexion with one feature of the interpretation of the electron spin resonance spectra of the radical $HO_2C{\cdot}CH_2{\cdot}CH{\cdot}\dot{C}O_2H$ in succinic acid crystals,[1,2] the authors prepared and irradiated *OO′*-dideuteriosuccinic acid in a single crystal with $^{60}Co$ γ-rays. The expected radical, $DO_2C{\cdot}CH_2{\cdot}\dot{C}H{\cdot}CO_2D$, was indeed formed and showed the same spectrum as its hydrogen analogue with modified line width and " spin flip " transitions.[2] Unexpectedly the spectrum contained additional lines which can unambiguously be attributed to the radical $DO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2D$ or its *O*-hydrogen analogues.

At first it was thought that *C*-deuteration had occurred during crystal growth from $D_2O$. The simple conditions were not such as were expected to give deuteration at the carbon atom [3] and a wide range of exchange conditions led to the same spectrum. Thus the $DO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2D/DO_2C{\cdot}CH_2{\cdot}\dot{C}H{\cdot}CO_2D$ ratio remained about 1 : 3 no matter whether the crystal was grown in 1 hour or in two days. Likewise, refluxing the acid in $D_2O$ for any length of time up to 10 days at pH's from 1 to 13 led to the same spectra. No evidence for $HO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2H$ could be found in the spectrum of any sample rapidly recrystallized from $H_2O$ no matter what its previous history of refluxing or crystallization from $D_2O$. Although the C–D stretching region in the infrared is obscured by O–D stretching frequencies, spectra from 4000 to 700 $cm.^{-1}$ supported the structure $DO_2C{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2D$, and in particular the infrared spectra confirmed that exchange of D with H occurred more rapidly than recrystallization to powder form from $H_2O$ could be carried out. The present authors are therefore convinced that the spectra were obtained after irradiating a crystal of pure *OO′*-dideuteriosuccinic acid without *C*-deuterated contaminant.

The most remarkable feature of the electron resonance spectra is the absence of any line which could be attributed to $DO_2C{\cdot}CH_2{\cdot}\dot{C}D{\cdot}CO_2D$ or to an isomeric form of $DO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2D$. In the crystalline succinic acid the two hydrogen atoms of the $CH_2$ group are distinguishable [4] and it is only hydrogen in one position, namely, that with the larger hyperfine coupling,[1,2] which is replaced. The Figure shows the spectrum for one orientation and indicates the position of lines to be expected if the other hydrogen

[1] Heller and McConnell, *J. Chem. Phys.*, 1960, **32**, 1535.
[2] Pooley and Whiffen, *Mol. Phys.*, 1961, **4**, 81.
[3] Ingold, " Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, 1953.
[4] Morrison and Robertson, *J.*, 1949, 980.

atoms were replaced by deuterium. It can be seen that the case is favourable for detection of these species, which appear to be entirely absent. It is estimated that if present to one-fifth of the concentration of the isomer which is present they would certainly be apparent, and at one-tenth the concentration they would still distort the equal-intensity deuterium triplet. Other crystal orientations gave spectra which support this conclusion. Irradiation at 90°K and observation at this temperature without intermediate warming led to entirely different spectra with the regular radicals absent. The new spectra were of a three-line pattern, with 1 : 2 : 1 relative intensity, with a hyperfine separation of about




Derivative of electron spin resonance absorption at 9200 Mc./sec. for irradiated *O,O'*-dideuteriosuccinic acid measured at 300° K. Field parallel to *Z'*, *i.e.* *c**. (i) line positions for $DO_2C{\cdot}CH_2{\cdot}\dot{C}H{\cdot}CO_2D$ present to about 75%. (ii) Line positions for $DO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2D$, with D replacing hydrogen of greatest coupling, present to 25%. (iii) Line positions for other isomer of $DO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2D$ or for $DO_2C{\cdot}CH_2{\cdot}\dot{C}D{\cdot}CO_2D$. For this special crystal orientation, these would give identical spectra. Both are absent.

25 Mc./sec., and with rather numerous weaker lines outside the main spectrum. The dominant spectral feature, which is the same for succinic as for *OO'*-dideuteriosuccinic acid, is tentatively assigned to $DO_2C{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}O{\cdot}$, but a full investigation has not been attempted.

The stereospecificity of the formation of $DO_2C{\cdot}CHD{\cdot}\dot{C}H{\cdot}CO_2D$ is even more puzzling than the occurrence of this radical, since its formation must occur rapidly below room temperature and is probably without appreciable activation energy. It may be that an internal radical or ion rearrangement is occurring. Alternatively the radical might be formed by attack by D or $D^+$ when the incoming particle might be confined to certain geometrical paths by virtue of the crystal structure. The results merely require that deuterium is sometimes incorporated and that this can occur in only one of the sites.

Although the authors are unable to suggest a convincing explanation of their observation, the result is reported at this stage as being of general interest and as a warning that deuteration studies can be misleading when used to help identification of free radicals by electron spin resonance techniques.

This work was carried out as part of a joint programme between the Chemistry Department, Birmingham University, and the Basic Physics Division, National Physical Laboratory. We thank Professor M. Stacey, F.R.S., Professor J. C. Robb, and Dr. J. K. Brown of the Department for their interest. D. P. acknowledges receipt of a University Scholarship.

THE CHEMISTRY DEPARTMENT, THE UNIVERSITY, BIRMINGHAM, 15.
BASIC PHYSICS DIVISION, NATIONAL PHYSICAL LABORATORY,
TEDDINGTON, MIDDLESEX. [*Received, August 4th*, 1961.]

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## 64. *The Chemistry of Colour Reactions: The Zimmermann Reaction.*

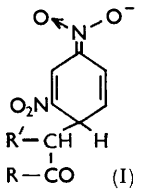
By T. J. KING and C. E. NEWALL.

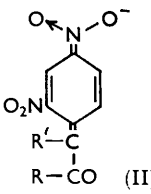
THE Zimmermann colour test [1] (methylene ketone, *m*-dinitrobenzene, alkali), which in essence is the same reaction as the Janovsky [2] test for *m*-dinitrobenzene, has been widely

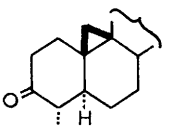
[1] Zimmermann, *Z. physiol. Chem.*, 1935, **233**, 257; 1936, **245**, 47.
[2] Janovsky and Erb, *Ber.*, 1886, **19**, 2155; Janovsky, *Ber.*, 1891, **24**, 971.

used for the detection and sometimes for the estimation of the group $-CO\cdot CH_2-$ in compounds, particularly, of the steroid and triterpene type. It shows some specificity in that it is negative for 6-, 7-, and 12-ketones in sterols [3,4] and it has been claimed [5] to be diagnostic of the presence of a 3-keto-group in pentacyclic triterpenes.

The original mechanism suggested [1] for the reaction was essentially addition of the activated methylene group to dinitrobenzene to give a chromophore of structure (I). This mechanism has not apparently been questioned until very recently when Zimmermann and his co-workers [6] suggested that an oxidation step was also involved in the test to give a chromophore of structure (II). We have also arrived at this conclusion on different evidence.


(I)


(II)


(III)

We originally considered that the formation of a chromophore of type (II) was better able to account for the reaction than the formation of a structure of type (I) because such a formula explains the requirement of a methylene group adjacent to carbonyl for a positive reaction, and it accounts for Reissert's observation,[7] which we have confirmed, that *m*-nitroaniline is formed during the test. Furthermore, spectroscopic examination of the coloured solutions produced in the test shows that the maximum absorption in the visible region is shifted to longer wavelength by the presence, in R (formulæ I and II), of unsaturation which is conjugated with the carbonyl group, an observation readily understandable on the basis of structure (II) but not of structure (I). If the coloured salt is of type (II) then acid should give the original ketone substituted in the α-position by 2,4-dinitrophenyl. Conversely, a 2,4-dinitrobenzyl ketone should dissolve in alkali to give the same colour as is produced during the Zimmermann test on the appropriate simple ketone.

2,4-Dinitrobenzyl phenyl ketone [8] has been known for some time and was originally reported to give a strong red-violet colour with alkali. We have examined the absorption spectrum of a solution of this ketone in dilute alcoholic alkali and find that it is virtually identical with the spectrum given by a solution of acetophenone and *m*-dinitrobenzene in alkali of the same concentration. Further, the intensity of the characteristic peak at 505 mμ was a maximum immediately the ketone had been mixed with alkali; in contrast, the intensity of the absorption band in a mixture of acetophenone, *m*-dinitrobenzene, and alcoholic potassium hydroxide increased steadily with time, up to a maximum at about 4½ hr. when it corresponded to the conversion of about 15% of the acetophenone into the coloured salt (II; R = Ph).

Zimmermann and his co-workers [6] isolated and analysed the coloured salt produced in his test from androsterone. In a similar way we isolated the coloured substance produced from acetophenone and, although the salt appeared to be unstable and was not analysed, treatment of it with acid followed by careful purification led to pure 2,4-dinitrobenzyl phenyl ketone. The positive demonstration of the formation of this compound, known to produce a colour with alkali indistinguishable from that produced in the test, can leave little doubt that the mechanism, envisaged by us and recently also advocated by Zimmermann, is correct.

[3] Kaziro and Shimada, *Z. physiol. Chem.*, 1937, **249**, 220.
[4] Callow, Callow, and Emmens, *Biochem. J.*, 1938, **32**, 1312.
[5] Barton and de Mayo, *J.*, 1954, 887.
[6] Neunhoeffer, Thewalt, and Zimmermann, *Z. physiol. Chem.*, 1961, **323**, 116.
[7] Reissert, *Ber.*, 1904, **37**, 831.
[8] Borsche, *Ber.*, 1909, **42**, 611.

The coloured compounds produced in the test are not indefinitely stable in alcoholic alkali. When 2,4-dinitrobenzyl phenyl ketone is dissolved in alkali about 25% of it can be recovered after 15 hr. The colour produced in the test is less permanent than these figures would suggest, possibly owing to the presence of an excess of *m*-dinitrobenzene and its reduction products. The permanence of the colour is also a function of the ketone used, as has been noted for some steroidal ketones;[4] the colour produced by acetone becomes brown after only one hour. The loss of the colour is much faster in the presence of appreciable concentrations of water.

A survey of the absorption spectra of the coloured solutions from a small number of different ketones suggests that the position of the maximum may have structural significance. Thus two acyclic ketones in which a methyl group must have been involved in the reaction gave solutions with $\lambda_{max.}$ 482 and 488 mμ. Conjugation of the ketone with a double bond or benzene ring extended this range to *ca.* 500 mμ (1 double bond) or 505 mμ (1 phenyl group). When $CH_2$ next to carbonyl in acyclic compounds is involved in the reaction the absorption maximum lies at a distinctly longer wavelength, *ca.* 535 mμ. Simple cyclic ketones showed a peak position which is sensitive to ring size, *e.g.*, 515 mμ for cyclopentanone, 525 mμ for cyclohexanone, 543 mμ for cycloheptanone. Unfortunately this correlation cannot be extended to include the more interesting polycyclic systems because in conformationally rigid structures the maximal absorption of the test solution falls at considerably shorter wavelength, *e.g.*, 467 mμ for cycloeucalenone[9] (partial structure III) and even the αβ-unsaturated ketone cholest-4-en-3-one affords a solution with a maximum at only 507 mμ.

*Experimental.*—Visible absorption measurements were made with a Unicam S.P. 600 spectrophotometer.

*Isolation of* m-*nitroaniline from a Zimmermann test.* A mixture of acetone (5 c.c.), *m*-dinitrobenzene (2 g.), and potassium hydroxide (1 g.) in methanol (50 c.c.) was added to water and steam distilled. Ether extracted *m*-nitroaniline (10 mg.), m. p. 113—114°, from the distillate. No *m*-nitroaniline was present in the steam distillate from solutions containing only *m*-dinitrobenzene, methanol, water, and potassium hydroxide, or *m*-dinitrobenzene, water, methanol, and acetone.

*Isolation of 2,4-dinitrobenzyl phenyl ketone from the Zimmermann test on acetophenone.* A solution of acetophenone (1·2 g.) and *m*-dinitrobenzene (3·5 g.) in ether (100 c.c.) was treated under nitrogen with methanol (50 c.c.) in which sodium (1 g.) had been dissolved. After 10 min. ether (1 l.) precipitated the red salt which was collected under nitrogen and washed with ether and benzene. The precipitate was then added to methanol (50 c.c.) containing sulphuric acid (5 c.c.), the mixture was diluted with water (100 c.c.), and most of the methanol was evaporated under reduced pressure at 50°. The aqueous residue was extracted with chloroform, and the extract was chromatographed on alumina (Spence's grade H) from which benzene eluted *m*-dinitrobenzene and chloroform eluted a red-brown band. The chloroform eluate was further purified on acid silica gel[10] from which benzene eluted a yellow band which afforded, by crystallisation from ethanol, colourless needles of 2,4-dinitrobenzyl phenyl ketone, m. p. and mixed m. p. 136—137°. The identity was further confirmed by the infrared absorption.

*Determination of* $\lambda_{max.}$ *for the Zimmermann test colours formed by a selection of ketones.* The ketone (2—5 mg.) and *m*-dinitrobenzene (3 mg.) were dissolved in 0·25N-ethanolic potassium hydroxide (10 c.c.) and the positions of the absorption maxima were determined after 1 hr.

We thank the Department of Scientific and Industrial Research for a grant (to C. E. N.).

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NOTTINGHAM. [*Received, August 11th*, 1961.]

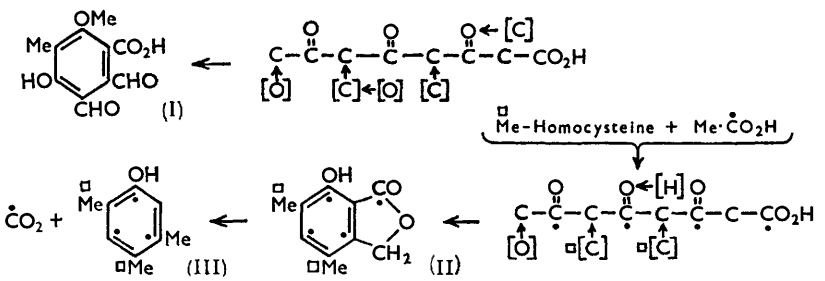
[9] Cox, King, and King, *J.*, 1959, 514.
[10] Brockmann, *Chem. Ber.*, 1958, **91**, 779.

## 65. *Studies in Relation to Biosynthesis. Part XXVI.* 7-Hydroxy-4,6-dimethylphthalide.*

By A. J. Birch and E. Pride.

It has been suggested [1] that *C*-methylation would be found to occur as a biochemical process, the methyl group coming from methionine. This was proved by tracer experiments to be true for *inter alia*, mycophenolic acid,[1] citrinin,[2,3] sclerotiorin,[2] ergosterol,[3] Terramycin.[4] Several examples are to be found of compounds derived partly from acetic acid where it is not easy, by inspection of the formula, to tell which atoms are derived from this source and which from methionine. One such case is cyclopaldic acid (I) where evidence was obtained, from incorporation of acetic acid only, that the biogenesis is probably as shown. We now report degradations of a similar compound, 7-hydroxy-4,6-dimethylphthalide (II) which support the expected biosynthetic origin shown below. The origins of the structures in this molecule are more obvious than in the acid (I) because of the lower degree of oxidation. Formation of the phthalide ring as a result of oxidation of a carbon biogenetically derived from a methyl of acetic acid is observed with other compounds, *e.g.*, mycophenolic acid.[1]

The results of [$^{14}$C]formic acid incorporation were clear-cut, although it was not possible to examine the two methyl groups separately. Kuhn–Roth oxidation of the substance




(r.m.a. 238·5) gives acetic acid, converted into acetone (2,4-dinitrophenylhydrazone r.m.a. 231) and carbon dioxide (r.m.a. substantially zero). The only activity incorporated was therefore in the methyl groups.

Incorporation of [1-$^{14}$C]acetic acid, and decarboxylation and reduction of the phthalide (II) (r.m.a. 152) by hydriodic acid and phosphorus, produced carbon dioxide (r.m.a. 42·6), the radioactivity of which was rather higher than expected (calc. 38). The other product, 2,4,5-trimethylphenol (III) (r.m.a. 104·5) contains an extra *C*-methyl group, from reduction of the lactone ring, which should, on the biosynthesis proposed, be attached to a labelled carbon atom. Kuhn–Roth oxidation of the phenol (III) gave acetic acid, converted [5] into acetone (r.m.a. of 2,4-dinitrophenylhydrazone, 11·5) and carbon dioxide (r.m.a. 11·4). Since Kuhn–Roth oxidation of the phthalide (II) itself gave almost inactive acetic acid (r.m.a. 0·5 as the *p*-phenylphenacyl ester) the other two *C*-methyl groups are attached as expected to inactive carbon atoms. If we assume equal yields of acetic acid from the three *C*-methyl groups of the phenol (III) the calculated r.m.a. for the carboxyl group on

* Part XXV, *J.*, 1961, 303.

[1] Birch, Elliott, and Penfold, *Austral. J. Chem.*, 1954, **7**, 169; Birch, English, Massy-Westropp, Slaytor, and Smith, *J.*, 1958, 365; *Chem. and Ind.*, 1957, 204.
[2] Birch, Fitton, Pride, Ryan, Smith, and Whalley, *J.*, 1958, 4576.
[3] Alexander, Gold, and Schwenk, *J. Amer. Chem. Soc.*, 1957, **79**, 2967, 4554.
[4] Snell, Birch, and Thomson, *J. Amer. Chem. Soc.*, 1960, **82**, 2402.
[5] Birch and Kocor, *J.*, 1960, 866.

the basis of one label to three molecules is 11·6 (*i.e.*, one-ninth of the r.m.a. of (III), 104·5, which itself contains 3 labels).

The slight excess of labelling on the lactone-carbonyl group is not at present explicable, but the overall results are clearly in accord with the postulated scheme.

*Experimental.—Penicillium gladioli* usually produces the related substance gladiolic acid, the phthalide (II) being a minor product. Its identity was confirmed by a mixed m. p. and infrared comparison with an authentic specimen kindly presented by Mr. J. F. Grove (Imperial Chemical Industries Limited, Akers Laboratories, Welwyn). Our strain of the mould must therefore have a slightly different metabolism from that previously studied.

*P. gladioli* (McCull and Thom) was grown on a simplified Raulin–Thom medium (7·5% of glucose) of initial pH 5 for 14 days. After 7 days [1-$^{14}$C]sodium acetate (0·05 mC) was added to each of two flasks and [$^{14}$C]sodium formate (0·05 mC) to each of two others. After a further 7 days the phthalide was extracted in the known [6] manner. Final purification was achieved by column elution from Florex with 1 : 1 benzene–light petroleum (b. p. 40—60°). From each of the above cultures about 60 mg. were obtained, which was diluted with inactive substance (200 mg.) and crystallised to constant radioactivity from methanol, then having m. p. 164—166° undepressed on admixture with 7-hydroxy-4,6-dimethylphthalide.

The apparatus used was that previously described [7] and the degradations were carried out as described in the literature [6] or by standard processes (see previous Parts of this series). 2,4,5-Trimethylphenol, m. p. 70—71°, was measured directly or as its 3,5-dinitrobenzoate, m. p. 175—176°.

The relative molar activities (r.m.a.) given above were calculated as previously described [7] ($\times 10^{-3}$).

We are indebted to Dr. Herchel Smith for discussions and to D.S.I.R. for a Scholarship (E. P.).

Department of Chemistry, University of Manchester. [*Received, August 29th*, 1961.]

[6] Raistrick and Ross, *Biochem. J.*, 1952, **50**, 635. Grove, *ibid.*, 1952, **50**, 648.
[7] Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360.

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## 66. *Reduction of Aromatic Nitro-compounds by Sodium Borohydride Catalysed by Palladised Charcoal.*

By Thomas Neilson, H. C. S. Wood, and A. G. Wylie.

Aromatic nitro- and nitroso-compounds are not normally reduced by sodium borohydride in aqueous or alcoholic solution.[1] Recently, in a preliminary communication,[2] Vlček and Rusina have described the use of cobalt–bipyridyl complexes as catalysts for this reaction. We have now discovered that a wide range of aromatic nitro- and nitroso-compounds are rapidly and smoothly reduced to the corresponding amines by sodium borohydride with a more convenient catalyst, palladised charcoal.

The nitro-compounds listed in the Table were reduced in each case to very pure amine in yields averaging 70%. The reduction was carried out in alkaline solution or in aqueous methanol, and the product was isolated as the free amine or, if more convenient, as benzoyl derivative. The rate of reduction was roughly proportional to the amount of catalyst added. With 0·01 mole of nitro-compound and 50 mg. of 10% palladised charcoal, the reduction was complete in 5—6 minutes at room temperature. The method appears superior to the " transfer hydrogenation " of aromatic nitro-compounds with hydrazine

[1] Gaylord, " Reduction with Complex Metal Hydrides," Interscience Publ. Inc., New York, 1956, p. 776.
[2] Vlček and Rusina, *Proc. Chem. Soc.*, 1961, 161.

and palladised charcoal,[3] which is only applicable to nitro-derivatives of the more reactive hydrocarbons.

We have been unable to isolate any intermediate compounds in the reduction, and there is no evidence that any by-products of the azoxybenzene, azobenzene, or hydrazobenzene type are formed and subsequently reduced. Indeed we have shown that azobenzene is reduced by this system to hydrazobenzene (isolated as benzidine) and not to aniline.

Palladised charcoal appears to have no catalytic effect on the reduction of aromatic nitro-compounds by lithium aluminium hydride. Thus, nitrobenzene is reduced to azobenzene both in the absence and in the presence of palladised charcoal.

| Nitro-compound | Product | M. p. of product: Amine | M. p. of product: Bz deriv. | Yield (%) |
|---|---|---|---|---|
| Nitrobenzene | Aniline | | 163—164° | 85 |
| *o*-Nitrophenol | *o*-Aminophenol | 167—169° | | 79 |
| *m*-Nitrophenol | *m*-Aminophenol | | 173—174 | 61 |
| *p*-Nitrophenol | *p*-Aminophenol | | 234 | 69 |
| *o*-Nitrobenzoic acid | Anthranilic acid | 144—145 | | 65 |
| *m*-Nitrobenzoic acid | *m*-Aminobenzoic acid | 173 | | 52 |
| *p*-Nitrobenzoic acid | *p*-Aminobenzoic acid | 184 | | 34 |
| *o*-Nitroaniline | *o*-Phenylenediamine | | 301 | 51 |
| *m*-Nitroaniline | *m*-Phenylenediamine | | 241 | 83 |
| *p*-Nitroaniline | *p*-Phenylenediamine | | >300 [a] | 71 |
| *p*-Nitrotoluene | *p*-Toluidine | | 158 | 76 |
| 1-Nitronaphthalene | 1-Naphthylamine | | 161—162 | 93 |
| Et *p*-nitrobenzoate | Et *p*-aminobenzoate | | 147—148 | 67 |
| 2,4-Dinitroaniline | 1,2,4-Triaminobenzene | | 260 | 79 |
| 2,4-Dinitrophenol | 2,4-Diaminophenol | | 253 | 60 |
| *m*-Dinitrobenzene | *m*-Phenylenediamine | | 241 | 81 |
| Dimethyl-*p*-nitrosoaniline | *p*-Aminodimethylaniline | | 226 | 93 |
| Azobenzene | Hydrazobenzene | | 352 [b] | 61 |

[a] The infrared spectrum of this material was identical that of with an authentic sample. [b] Isolated as dibenzoylbenzidine.

*Experimental.*—The following procedure is typical. 10% Palladised charcoal (50 mg.) was suspended in water (10 ml.), and sodium borohydride (0·78 g., 0·02 mole) in water (15 ml.) was added. A slow stream of nitrogen was bubbled through the mixture and a solution of *o*-nitrophenol (1·39 g., 0·01 mole) in 2N-sodium hydroxide solution (50 ml.) was added dropwise during 5 min. The mixture was left at room temperature for a further 10 min. After filtration, the solution was acidified to destroy the excess of borohydride, and the product was extracted with ether (3 × 50 ml.) from the neutralised solution. Evaporation of the dried ethereal extract gave *o*-aminophenol (0·87 g., 79%), m. p. 167—169°, mixed m. p. 168—170°.

When the nitro-compound is insoluble in aqueous solution it may be conveniently dissolved in methanol. We recommend that the reagents be added in the order given above. This prevents the possible ignition of hydrogen which can take place on addition of dry palladised charcoal to solutions of sodium borohydride.

The rate of reduction was shown to be roughly proportional to the amount of catalyst added by the following experiment. *o*-Nitrophenol (0·01 mole) in alkali was added all at once to a mixture of sodium borohydride (0·02 mole) and 10% palladised charcoal. The time taken for disappearance of the yellow colour of the nitro-compound in alkali was noted:

| | | | | | |
|---|---|---|---|---|---|
| Amount of catalyst (mg.) ..... | 200 | 100 | 50 | 30 | 10 |
| Time (min.) ..... | 1·5 | 3 | 6 | 10 | 80 |

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GLASGOW, C.1.

[*Received, September 6th*, 1961.]

[3] Dewar and Mole, *J.*, 1956, 2556.

## 67. *An Improved Preparation of Methyl 3-Amino-3-deoxy-α-D-mannoside Hydrochloride.*

By A. C. Richardson.

The preparation of methyl 3-amino-3-deoxy-α-D-mannoside from methyl α-D-glucoside has been described recently by Baer and Fischer.[1] The glucoside was oxidised with sodium metaperiodate, and the dialdehyde so formed was cyclised with nitromethane in the presence of base to a mixture of 3-*aci*-nitropyranosides in the form of sodium salts. "Dry" acidification of this mixture with sodium hydrogen sulphate yielded a mixture of 3-nitropyranosides which upon hydrogenation gave a 22—27% overall yield of the mannoside. This procedure, however, involves several stages and requires relatively large quantities of platinum dioxide as the hydrogenation catalyst; the undoubted usefulness of this reaction in the large-scale preparation of this important synthetic intermediate is thus somewhat impaired.

Modifications have been made which have considerably simplified and facilitated the preparation on a large scale. Isolation of the intermediate products was unnecessary, the *aci*-nitro-salts could be converted into the nitro-derivatives by cation-exchange resin, and Raney nickel was a better catalyst [2] for the final hydrogenation; the mannoside is now readily obtained in a yield of 20—23%.

The mannoside was further characterised by the formation of a tetra-acetyl derivative, which with methanolic ammonia afforded methyl 3-acetamido-3-deoxy-α-D-mannoside.[3]

*Experimental.—Methyl 3-amino-3-deoxy-α-D-mannoside hydrochloride.* Sodium metaperiodate (220 g.) was slowly added to a stirred solution of methyl α-D-glucoside (100 g.) in water (500 ml.) at $<20°$ (portionwise addition of ice). The mixture was then stirred for 1 hr., and the released formic acid neutralised with sodium hydrogen carbonate (40 g.). The mixture, from which much sodium iodate had crystallised, was then poured into ethanol (500 ml.) and the precipitated salts were filtered off. The filtrate was concentrated to a thin syrup, which was extracted with ethanol (800 ml.). After filtration, the ethanolic extract was treated with nitromethane (60 ml.), followed by a solution from sodium (12 g.) in methanol (600 ml.). The mixture was kept at room temperature for 20 min., then neutralised with Amberlite IR-120(H) resin (*ca.* 700 ml.). After removal of the resin the solution was concentrated to a syrup, which was extracted with hot ethyl acetate (400 ml.). A small amount of insoluble material was removed by filtration through Hyflo Supercel, and the extract concentrated to a wine-red syrup. A solution of this syrup in ethanol (*ca.* 300 ml.) was hydrogenated with Raney nickel-T4 catalyst [4] (*ca.* 10—20 g.), at an initial pressure of 4 atm. When the reduction was complete (1—8 hr.), the catalyst was filtered off and the filtrate treated with concentrated hydrochloric acid (30 ml.; *ca.* 0·75 equiv.) whereupon crystallisation of the mannoside salt commenced. After 1 hr. at 0°, methyl 3-amino-3-deoxy-α-D-mannoside hydrochloride (24·8 g., 21%) was filtered off. It had $[\alpha]_D$ +60° (*c* 2 in $H_2O$) and decomposed without melting at 210—240° (Baer and Fischer [1] report $[\alpha]_D$ +60° and decomposition at *ca.* 205°) [Found: C, 36·8; H, 6·8; N, 6·0%; *M*, 227 (potentiometric titration). Calc. for $C_7H_{16}ClNO_5$: C, 36·6; H, 7·0; N, 6·1%; *M*, 229·7].

*Methyl 3-acetamido-2,4,6-tri-O-acetyl-3-deoxy-α-D-mannoside.* A mixture of the amino-mannoside hydrochloride (3·9 g.), acetic anhydride (60 ml.), and pyridine (60 ml.) was kept at room temperature for 4 hr. with occasional shaking. The excess of anhydride was then decomposed with ice and the solution concentrated to a crystalline residue. Recrystallisation from ethanol afforded 4·7 g. (77%) of *methyl 3-acetamido-2,4,6-tri-O-acetyl-3-deoxy-α-D-mannoside*, m. p. 153°, $[\alpha]_D$ +41° (*c* 1·75 in $H_2O$) (Found: C, 49·6; H, 6·5; N, 3·8. $C_{15}H_{23}NO_9$

[1] Baer and Fischer, *J. Amer. Chem. Soc.*, 1960, **82**, 3709.
[2] Richardson and Fischer, *J. Amer. Chem. Soc.*, 1961, **83**, 1132.
[3] Saltza, Reid, Dutcher, and Wintersteiner, *J. Amer. Chem. Soc.*, 1961, **83**, 2785.
[4] Nishimura, *Bull. Chem. Soc. Japan*, 1959, **32**, 61.

requires C, 49·85; H, 6·4; N, 3·9%). Like other acetylated amino-sugar derivatives, this compound was soluble in water (cf. Coxon and Hough [5]).

*Methyl 3-acetamido-3-deoxy-α-D-mannoside.* The tetra-acetyl derivative (5·35 g.) was dissolved in methanol (60 ml.) and aqueous ammonia (60 ml.; *d* 0·88) and kept at room temperature for 18 hr. Concentration afforded crystals, which were recrystallised from ethanol to give the *N*-acetyl derivative, m. p. 241—243°, $[\alpha]_D$ +44° (*c* 1·66 in $H_2O$) (2·75 g., 80%) (Found: C, 45·6; H, 7·4; N, 5·95. Calc. for $C_8H_{17}NO_6$: C, 45·95; H, 7·3; N, 5·95%). Saltza *et al.*[3] report m. p. 242·5—243·5° and $[\alpha]_D$ +17°, the latter being at variance with our value.

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[5] Coxon and Hough, *J.*, 1961, 1463.

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## 68. *The Use of Triethylamine in the Preparation of* N-*Carboxy*-L-*proline Anhydride.*

By A. A. RANDALL.

BERGER, KURTZ, and KATCHALSKI [1] reported the first successful synthesis of *N*-carboxy-L-proline anhydride. *N*-Benzyloxycarbonyl-L-proline was converted into the anhydride by treatment with phosphorus pentachloride in benzene solution. However, the yield was poor and purification of the product was difficult. Kurtz *et al.*[2,3] later described an improved method involving the use of carbonyl chloride and silver oxide. The intermediate *N*-chloroformyl-L-proline formed by the reaction in dioxan was cyclised to *N*-carboxy-L-proline anhydride by means of silver oxide in acetone. The experimental procedure has now been further simplified and the yield of product increased by replacing silver oxide by triethylamine. The intermediate chloroformyl derivative is cyclised to the anhydride *in situ* by addition of an equivalent amount of triethylamine.

Triethylamine is superior to other tertiary organic bases (*e.g.*, pyridine, tri-n-butylamine) because of its reactivity and the ease with which its hydrochloride crystallises from the solution.

*Experimental.*—Commercial dioxan was purified by Vogel's procedure.[4]

Triethylamine was refluxed with acetic anhydride (10% v/v), fractionally distilled under a vacuum, kept over sodium hydroxide for 20 hr., redistilled under a vacuum from the desiccant, and finally fractionated *in vacuo*.

N-*Carboxy*-L-*proline anhydride.* A colloidal suspension of L-proline (20 g.) in dioxan (100 ml.) was slowly added, together with a solution of carbonyl chloride (18 g.) in dioxan (100 ml.), to dioxan (200 ml.). The mixture was stirred until a clear solution was obtained (~1 hr. at 45°). After filtration from traces of reactant, the solution was degassed *in vacuo* at 35° for about 45 min. The amount of *N*-chloroformyl-L-proline formed was estimated by titrating 1 ml. of this solution with 0·1N-silver nitrate. Triethylamine (23·4 ml.) was added, and the whole stirred for 30 min. at room temperature. Precipitated triethylamine hydrochloride was removed and the filtrate concentrated *in vacuo* to a colourless oil that was triturated with light petroleum until it crystallised, and then collected (21·5 g., 88%). Vacuum sublimation or recrystallisation from ethyl acetate gave white crystals, m. p. 45° (decomp.) (Found: C, 51·0; H, 5·0; N, 9·9. Calc. for $C_6H_7O_3N$: C, 51·1; H, 5·0; N, 9·9%).

Pure *N*-carboxy-L-proline anhydride in pyridine gave a polymer which after precipitation

[1] Berger, Kurtz, and Katchalski, *J. Amer. Chem. Soc.*, 1954, **76**, 5552.
[2] Kurtz, Berger, and Katchalski, *Nature*, 1956, **178**, 1066; Kurtz, Berger, and Katchalski, "Recent Advances in Gelatin and Glue Research," Pergamon Press, London, 1958, p. 131.
[3] Kurtz, Fasman, Berger, and Katchalski, *J. Amer. Chem. Soc.*, 1958, **80**, 393.
[4] Vogel, "A Text-book of Practical Organic Chemistry," Longmans, Green and Co., London, p. 175.

by ether and redissolution in acetic acid underwent mutarotation. This reaction, characteristic of the transformation poly-L-proline I ⟶ II, was similar to that which has been described earlier.[2,5]

A procedure, identical with that described above, may be used for the preparation of 4-acetyl-*N*-carboxy-L-proline anhydride from 4-acetoxy-L-proline.

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[*Received, July 31st*, 1961.]

[5] Downie and Randall, *Trans. Faraday Soc.*, 1959, **55**, 2132.

## 69. *The Chelates formed by Tin(II) with Ethylenediaminetetra-acetic Acid.*

By H. G. LANGER and R. F. BOGUCKI.

A RECENT publication [1] by T. D. Smith on complexes of tin(II) with ethylenediaminetetra-acetic acid (EDTA) prompts us to report some of our results and to draw attention to some ambiguities in Smith's paper and discrepancies between his and our findings.

On p. 2554, the statement is made, " When the molar ratio of tin(II) to EDTA disodium salt was 1 : 0·5, no precipitation occurred on titration. The chelate formed must be $Sn_2Y$, the reaction, $Sn^{2+} + \frac{1}{2}H_2Y^{2-} = \frac{1}{2}Sn_2Y + H^+$, leading to an inflexion at $a = 1$. It is assumed therefore that the chelate is readily hydrolysed." It is not clear from this whether the inflection for the titration of the 2 : 1 tin(II)–EDTA chelate occurs at $a = 1$ (where $a$ is the " number of equivalents of alkali added per g.-atom of metal ion ") as stated in the text or at $a = 2$ which seems to be indicated by the titration curve given. If the inflection was observed at $a = 1$, the assumption that the chelate is readily hydrolyzed is unjustified, since hydrolysis would require consumption of more alkali which, however, is not reported. On the other hand, if the inflection occurred at $a = 2$ as indicated by the titration curve, this implies the quantitative formation of a soluble 2 : 1 hydroxo-chelate containing one hydroxyl group per metal but does not supply any direct evidence for the formation of $Sn_2Y$ which requires an inflection at $a = 1$. In this laboratory we have previously isolated the 2 : 1 tin(II)–EDTA chelate and identified it by analysis and by its infrared spectrum; some of its properties have been described.[2] In contrast to Smith's observation that no precipitation occurred on titration of the 2 : 1 tin(II)–EDTA system, we have in each case observed a substantial precipitation during titration of this system under varying conditions of concentration and ionic strength.

We have also investigated the reaction of stannous chloride with the disodium salt of EDTA in a 1 : 1 ratio. Titration of this system with base in the absence of excess of chloride ion and with rigorous exclusion of oxygen leads to a single steep inflection at $a = 2$. We found no evidence for the formation of the species $[SnY{\cdot}OH]^{3-}$ which is suggested by Smith. When the same system was titrated under an atmosphere of nitrogen in the presence of 0·1M-potassium chloride, but where precautions were not taken to exclude oxygen from all preparatory steps, we obtained a curve similar to the reported 1 : 1 titration curve. It may be significant that the reported 1 : 1 tin(II)–EDTA curve is also similar to our titration curve for the 1 : 1 tin(IV)–EDTA chelate, for which compound we have definite evidence for the formation of a hydroxo-chelate.

The use of polarographic measurements at pH 4·5 for tin(II) analysis is suggested (p. 2555) yet curves are not shown at pH 4·5, nor is the claim for the existence of $SnY^{2-}$ between pH 3·6 and 6·0 substantiated by polarographic or spectrophotometric measurements since the curves reported do not include data for these pH values.

[1] Smith, *J.*, 1961, 2554.
[2] Langer, *J. Dent. Res.*, 1960, **39**, 740.

In the discussion of the calculation of the stability constant for tin(II)–EDTA, no mention is made of the hydrolysis of the $Sn^{2+}$ and $Th^{4+}$ ions at pH 4. In fact, the Table (p. 2556) shows that essentially all of the tin in the solution exists as the two species $SnY^{2-}$ and $Sn^{2+}$. This is quite surprising since other studies [3] indicate that both $Sn^{2+}$ and $Th^{4+}$ ions would be hydrolyzed to a significant extent at pH 4. Therefore, a significant fraction of the non-chelated $Sn^{2+}$ and $Th^{4+}$ ions will exist at pH 4 as hydroxo-complexes which must be considered in evaluating the formation constant.

Thus, we have found that the Sn(II)–EDTA system in aqueous solution is complex and that special precautions must be taken in order to obtain reliable and reproducible results. It is to be hoped that the apparent discrepancies between our results and those of Smith can be explained by difference in experimental detail.

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[3] Tobias, *Acta Chem. Scand.*, 1958, **12**, 198; Hietanen, *Acta Chem. Scand.*, 1954, **8**, 1626; Kraus and Holmberg, *J. Phys. Chem.*, 1954, **58**, 325.