700. *Studies in Relation to Biosynthesis. Part XXXI.*[1] *Some Developments of the Bromopicrin Reaction.*

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Yields of bromopicrin in the hypobromite degradations of some aromatic nitro-compounds have been examined to define good general conditions for use in the investigation of ^{14}C-labelling patterns. Reduction of bromopicrin, even when impure, with iron powder gives methylamine, isolated as *N*-methyl-2,4-dinitroaniline. The method is used to examine the radioactivity of several individual carbon atoms in 6-methylsalicyclic acid derived biosynthetically from [2-^{14}C]acetic acid.

In studying the biosynthesis of aromatic compounds by radiotracer methods it is often desirable to isolate degradation products corresponding to a single position of the ring. Any method for this purpose should have the characteristics: (*a*) that the ring is degraded to give a small fragment not containing more than one or two carbon atoms; (*b*) that this fragment can be related unequivocally to the position it occupied in the ring; (*c*) that mixtures formed should be easily separable; and (*d*) that yields should be good. One very good example is the Kuhn–Roth oxidation, but this is limited to compounds carrying *C*-methyl groups. The most promising direct degradations of the rings, particularly of phenolic compounds, involve oxidative halogenation.[2] The exact nature of the products varies with the compounds and conditions, and the methods may have a limited use; we find, for example, that phloroglucinol under the conditions of the bromopicrin degradation below gives about one mol. of a mixture of bromoform and carbon tetrabromide, undoubtedly derived from the 2,4,6-positions.

The most attractive general method is the degradation of aromatic nitro-compounds by hypobromite, which fulfils most of the conditions above. This reaction has already been used for examining ^{14}C-distribution [3-5] but its generality has not been examined and it suffers from the disadvantage that the bromopicrin produced is an oil which is not readily purified. The reaction proceeds readily with polynitro-compounds, *e.g.*, 3-methyl-2,4,6-trinitrophenol,[3] trinitrophloroglucinol,[4] 2,4,6-trinitro-orcinol,[4] 4-hydroxy-3,5-dinitrobenzoic acid,[5] 2,4,6-trinitrophenol,[6] 2,4,6-trinitroresorcinol,[7] and 2,5-dihydroxy-3,6-dinitrobenzoquinone,[8] and the bromopicrin is readily separated by steam-distillation. It is frequently contaminated by bromoform and carbon tetrabromide. Sharp infrared

[1] Part XXX, *J.*, 1962, preceding paper.
[2] Cf. Beilstein, "Handbuch der Organische Chemie," Springer, Berlin, 1925, Vol. VII, 852; Vol. VIII, 376; 1931, Vol. VII, 468; 1931, Vol. VIII, 679; Booth and Saunders, *Chem. and Ind.*, 1950, **69**, 824.
[3] Birch, Massy-Westropp, and Moye, *Austral. J. Chem.*, 1955, **8**, 539.
[4] Birch, Massy-Westropp, Rickards, and Smith, *Proc. Chem. Soc.*, 1957, 98; *J.*, 1958, 360.
[5] Baddiley, Ehrensvärd, Klein, Reio, and Saluste, *J. Biol. Chem.*, 1950, **183**, 777.
[6] Stenhouse, *Annalen*, 1854, **91**, 307; Bolas and Groves, *J.*, 1870, **23**, 153.
[7] Lei and Sah, *Sci. Reports Nat. Tsinghua Univ.*, 1933, *A*, **2**, 129; *Chem. Abs.*, 1934, **28**, 97.
[8] Levy and Jedlicka, *Annalen*, 1888, **249**, 66.

maxima at 1145 cm.$^{-1}$ ($CHBr_3$), 1310, 838, and 800 cm.$^{-1}$ (bromopicrin), and 670 cm.$^{-1}$ (CBr_4) can be used for detection, but are less useful for precise analysis of a mixture. A further investigation has been carried out to define the scope and conditions of the reaction and to remove difficulties due to contamination.

Starting with the conditions used by Will,[9] but substituting barium hydroxide for calcium hydroxide, we examined a series of varied conditions. The most generally successful conditions (see Experimental section) were worked out for 2-methyl-4,6-dinitrophenol and then applied in other cases with a correction for the molecular weight and the number of nitro-groups present. This is a rather arbitrary procedure, and clearly the optimum conditions may well not result in many cases; the yields of volatile product were, however, usually good. They are shown in the Table, calculated as bromopicrin (mol.), with bromine analyses (calc., 80·6%).

Infrared examination of the products from 2,4,6-trinitro-orcinol and 3,5-dinitrobenzoic acid showed little trace of bromoform, whereas the products from *o*- and *p*-nitrophenol contained about 37% and 75%, respectively, of bromoform.

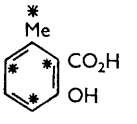
Several facts emerge: the more nitro-groups there are present, the higher is the yield per group and the greater the purity of the bromopicrin, and yields are somewhat depressed by methyl substituents. These results are probably related to the necessity for producing oxidised non-aromatic intermediates, which would be facilitated by the presence of phenolic or nitro-groups and rendered more difficult by blockage with methyl groups. Possibly 1,3,5-trinitrobenzene is slowly oxidised to picric acid; it is initially insoluble.

Compound	Yield (mol.) of volatile product	Br (%)	Compound	Yield (mol.) of volatile product	Br (%)
m-Nitrobenzenesulphonic acid	0	—	3,5-Dinitrobenzoic acid	1·5	84·1
3- or 4-Nitrophthalic acid	0	—	4-Methyl-3,5-dinitrophenol	1·4	—
p-Nitrobenzoic acid	Trace	—	2-Methyl-4,6-dinitrophenol	1·6	84·4
2,4-Dinitrotoluene	0	—	2,4,6-Trinitro-orcinol	2·6	81·3
p-Nitrophenol	0·43	90·3	2,4,6-Trinitrobenzoic acid	2·7	81·0
m-Nitrophenol	0·65	90·3	2,4,6-Trinitrophenol	2·9	—
o-Nitrophenol	0·58	87·1	*m*-Dinitrobenzene	Small	—
2,4-Dinitrophenol	1·4	84·4	1,3,5-Trinitrobenzene	Small	—
3,5-Dinitrophenol	1·3	84·6	1,3,5-Trinitrobenzene *	2·6	—

* With preliminary stirring for 3 hr. before heating.

In view of the occasional very great contamination of the bromopicrin, it is necessary to convert it into a crystalline derivative before ^{14}C-assay. This was done by reducing the mixture with iron powder and dilute hydrochloric acid, in a manner similar to that used[10] with chloropicrin, into methylamine, which was then converted into the highly crystalline *N*-methyl-2,4-dinitroaniline. The product obtained by this sequence in high yield from an artificial mixture of bromopicrin and bromoform was shown by melting point and infrared spectrum to be identical with a pure synthetic specimen.

In order to check the general method further, we have applied it to examine the degradation of 6-methylsalicylic acid (I) derived biosynthetically by growth of *Penicillium griseofulvum* in the presence of [2-^{14}C]acetic acid. From previous work with [1-^{14}C]acetate,[3,11] this metabolite should have the labelling pattern shown (I; * indicates ^{14}C). In view of recent results implicating malonic acid as an intermediate in this biosynthesis,[11,12] it is desirable to check if possible whether each biosynthetic C_2-unit has in fact the same radioactivity from the [2-^{14}C]acetate precursor.



(I)

[2-^{14}C]Acetate is normally incorporated with some randomisation of label through involvement in the tricarboxylic acid cycle, so it was necessary first to check the extent

[9] Will, *Ber.*, 1914, **47**, 962.
[10] Geisse, *Annalen*, 1859, **109**, 282; Frankland, Challenger, and Nicholls, *J.*, 1919, **115**, 161.
[11] Birch, Cassera, and Rickards, *Chem. and Ind.*, 1961, 792.
[12] Bu'Lock and Smalley, *Proc. Chem. Soc.*, 1961, 209.

of this. Decarboxylation of the methylsalicylic acid (r.m.a. $71{\cdot}0 \times 10^3$) gave carbon dioxide, assayed as barium carbonate (r.m.a. $0{\cdot}39 \times 10^3$), which indicated about 2% of randomisation. Nitration [13] of the *m*-cresol obtained therefrom by decarboxylation afforded 3-methyl-4-nitrophenol (r.m.a. $72{\cdot}3 \times 10^3$) and 3-methyl-6-nitrophenol (r.m.a. $68{\cdot}6 \times 10^3$), which were degraded by hypobromite to give *N*-methyl-2,4-dinitroaniline, of r.m.a. $17{\cdot}2 \times 10^3$ and $17{\cdot}7 \times 10^3$, respectively, in excellent agreement with the value $17{\cdot}4 \times 10^3$ to be expected on the basis of the randomisation noted and equal labelling in the asterisked carbons of the acid (I).

This work leads to the conclusion that a fairly general method now exists whereby the extent of ^{14}C-labelling of an aromatic position can be ascertained with accuracy provided a nitro-group can be attached to that position.

Experimental

Radioactive samples were assayed at infinite thickness in 0·3 cm.^{2} planchettes; for details see Part XIII of this series.[4]

Degradations to Bromopicrin.—2-Methyl-4,6-dinitrophenol (2·5 g.) was warmed with water (250 c.c.), and sufficient barium hydroxide was added to produce a solution. (In some other cases the barium salt produced a slurry, particularly on cooling, but this was used without difficulty provided the salt was efficiently formed.) This solution was cooled to 0° and added to a solution of bromine (21 g.) in water (1 l.) containing hydrated barium hydroxide (35 g.) at 0°. After being stirred for 30 min. the mixture was heated rapidly to the b. p., then the bromopicrin was rapidly steam-distilled. It was collected by removing the heavy layer and dried ($CaCl_2$) if necessary. Further small amounts could be collected by extraction with a little ether (total yield 1·58 mol.). The combined wet substance could be used directly for the reduction below.

In other cases the general conditions were the same, the amounts of reagents being varied directly as the number of nitro-groups and inversely as the molecular weight. In fact the yields were not found to be very sensitive to amounts of reagent provided that bromine in about the above proportion was used; they fell off markedly with increases of 30—100% in the proportion of barium hydroxide.

Reduction of Bromopicrin.—Bromopicrin (250 mg.) was shaken in a tightly stoppered flask with clean iron filings (500 mg.) and 0·1N-hydrochloric acid (10 c.c.) for 3 hr. The solution was then briefly steam-distilled, rendered alkaline with N-sodium hydroxide, and steam-distilled into 0·1N-hydrochloric acid through an adaptor leading below the surface of the acid solution. Evaporation to dryness left crystalline methylamine hydrochloride. This was refluxed with 1-chloro-2,4-dinitrobenzene (128 mg.) in ethanol (10 c.c.) for 15 min. with gradual addition of N-sodium hydroxide (1·25 c.c.). Addition of water produced a solid (70—80 mg.) which, when recrystallised from aqueous ethanol several times, gave *N*-methyl-2,4-dinitroaniline (55 mg.), m. p. 175—177° (Found: C, 42·9; H, 3·7. Calc. for $C_7H_7N_3O_4$: C, 42·6; H, 3·6%). Further purification of radioactive material to constant count-rate could be assisted, if necessary, by chromatography on " Florisil," the substance being adsorbed in a little acetone and eluted with ether, or by sublimation at 100—120°/0·05 mm.

Degradation of [^{14}C]-6-*Methylsalicyclic Acid.*—[^{14}C]-Methylsalicyclic acid (r.m.a. $71{\cdot}0 \times 10^3$) was produced as described [3] by feeding $^{14}CH_3{\cdot}CO_2Na$ to *Penicillium griseofulvum* Dierckx (L.S.H.T.M. strain P. 68) and was purified by recrystallisation from water and sublimation at 140°/0·05 mm.

Decarboxylation [3] of the acid (4·87 g.) gave *m*-cresol (3·30 g.) and carbon dioxide, assayed as barium carbonate (r.m.a. $0{\cdot}39 \times 10^3$). The *m*-cresol (3·30 g.) was nitrated as described in the literature,[13] to give 3-methyl-4-nitrophenol (50%), m. p. 129°, purified by sublimation at 100°/0·05 mm. and crystallised from benzene–light petroleum (r.m.a. $72{\cdot}3 \times 10^3$), and 3-methyl-6-nitrophenol (25%), m. p. 56° after sublimation at 20°/15 mm. and crystallisation from aqueous ethanol (r.m.a. $68{\cdot}6 \times 10^3$).

To 3-methyl-6-nitrophenol (199 mg.) in hot water (20 c.c.) was added hydrated barium hydroxide (1·5 g.) in hot water (40 c.c.). The salt suspension was cooled to 0° and added to

[13] Staedel and Kolb, *Annalen*, 1890, **259**, 208; Khotinsky and Jacopson-Jacopmann, *Ber.*, 1909, **42**, 3097.

barium hypobromite solution [from bromine (1·8 c.c.) and barium hydroxide (12·5 g.) in water (230 c.c.)]. After 1 hour's stirring at 0° steam-distillation gave an oil (218 mg.), shown by infrared analysis to be 90% pure bromopicrin. Reduction of the mixture as above gave *N*-methyl-2,4-dinitroaniline, m. p. 175—177°, after sublimation at 100—120°/0·05 mm. and crystallisation from aqueous ethanol (r.m.a. $17·7 \times 10^3$).

3-Methyl-4-nitrophenol (295 mg.), similarly treated, gave a steam-volatile oil (340 mg.), shown by infrared analysis to contain 55% of bromopicrin, which was converted into *N*-methyl-2,4-dinitroaniline (r.m.a. $17·2 \times 10^3$).

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