## 676. *A Radioactive Drug: 2-Methyl-6-tritio-1,4-naphthaquinol Bis(disodium Phosphate) and 2-Methyl-5,6,7-tritritio-1,4-naphthaquinol Bis(disodium Phosphate).*

By K. J. M. Andrews, F. Bultitude, E. A. Evans, M. Gronow, R. W. Lambert, and D. H. Marrian.

2-Methyl-6-tritio-1,4-naphthaquinol bis(disodium phosphate) has been prepared by reductive dehalogenation of the corresponding 6-iodo-compound by carrier-free tritium. The product, of specific activity 27 curies per millimole, remained essentially radiochemically pure in aqueous solution for several months. Preliminary laboratory and clinical investigations of the compound as a new form of treatment of human malignancies suggested the need for even higher activity. 5,6,7-Tribromo-2-methyl-1,4-naphthaquinol bis(disodium phosphate) was therefore synthesised and likewise reduced to the tritritio-compound, of specific activity 83 curies per millimole.

The investigations of compounds which increase the therapeutic effects of ionising radiations, which began in this Department in 1946, gave early and unexpected indications that the first compound studied, 2-methyl-1,4-naphthaquinol bis(disodium phosphate) (I; R = R′ = R″ = H), was selectively concentrated, under certain conditions, by tumour tissue. For example, after intravenous injections of its solutions into rats bearing the Walker 256 carcinoma, a fluorescence in ultraviolet light developed in certain organs after they had been treated with alkaline hydrogen peroxide. This fluorescence, which was spectrally identical with that shown by 2-methyl-1,4-naphthaquinone 2,3-oxide, seemed most intense about 30

(I)

minutes after the injection and was subjectively and photographically much stronger at the growing edge of the tumour than elsewhere; it was also apparent in organs concerned with detoxification and excretion.[1]

There followed attempts to confirm this effect by labelling the compound; although radioactive halogen substituents were tried,[2, 3] these proved labile under physiological conditions, as had the phosphorus label studied by Neukomm *et al.*[4] A carbon-labelled methyl group [2] did allow us, however, to confirm the uptake and retention in human tumours.[5, 6]

It thus appeared that we could use this molecule to carry ionising radiation to the growing edges of a tumour and, provided that a sufficient degree of radioactivity could be incorporated, a new form of cancer therapy might be possible. Tritium seemed the ideal isotope for this purpose since it is readily available in pure form and its low-energy β-emission [7] would ensure that only the cell in which the molecule was fixed would be affected by the radiation; hazards to the clinical staff would be negligible. These arguments have been fully developed in a recent review.[8]

So far, three methods have been used to incorporate tritium into the compound. The first, and simplest, was to allow 2-methyl-1,4-naphthaquinone to react with sodium hydrogen sulphite in the presence of tritiated water. Regeneration of the quinone and subsequent reduction and phosphorylation furnished the compound labelled in position 3 of the nucleus.[9] Although useful for tracer studies, the product had too low a specific activity for clinical use.

The second employed Wilzbach's method [10] whereby tritium is incorporated by exchange. Irradiation of the anhydrous salt (I; R = R′ = R″ = H) with tritium at room temperature for 14 days resulted in the incorporation of ~15% of the tritium used. However, it was found that, of the activity associated with the product, only about 7% was not removed by freeze-drying of an aqueous solution of the product. Dilution analysis of the generally labelled product, after removal of the labile tritium, showed that the radiochemical purity of the compound was only 11%. Ceric sulphate oxidation of the purified product to the corresponding quinone established that all the remaining tritium was firmly bound. In several irradiations so conducted, the specific activity of the purified salt (I; R = R′ = R″ = H) did not exceed 1 curie per millimole.

The most promising method of preparing material containing one or more tritium atoms per molecule in high yield seemed to be by reductive dehalogenation of suitable quinol bis-(disodium phosphates). Preliminary experiments showed that the 3-bromo-derivative (I; R = Br, R′ = R″ = H) [11] could be reduced catalytically in aqueous solution at atmospheric pressure and temperature and that, in a suitably designed high-vacuum system,[12] the uptake of hydrogen was theoretical and could involve very little loss of the reagent gas. Reduction in the presence of pure tritium gave a product containing 4·5 curies per millimole compared with the theoretical activity of 28 curies per millimole for complete replacement of one atom by tritium. It seemed obvious that exchange with the solvent was occurring, initiated by the mol. of $^3$H-hydrobromic acid necessarily produced. This was later avoided by altering the solvent [13] and by ensuring that the pH remained on the


[1] Mitchell, *Acta Radiologica*, 1953, *Suppl.* 116, 431.
[2] Andrews, Marrian, and Maxwell, *J.*, 1956, 1844.
[3] Marrian and Maxwell, *Brit. J. Cancer*, 1956, **10**, 739.
[4] Neukomm, Pequiron, Lerch, and Richard, *Arch. int. Pharmacolodyn.*, 1953, **93**, 373.
[5] Marrian and Maxwell, *Brit. J. Cancer*, 1956, **10**, 575.
[6] Horwitz, Gregg, Marrian, Marshall, and Mitchell, *Acta Radiologica*, 1959, *Suppl.* 188, 111.
[7] Gregory and Landsman, *Phys. Rev.*, 1958, **109**, 2091.
[8] Marrian, Marshall, and Mitchell, *Chemotherapia*, 1961, **3**, 225.
[9] Marrian, *J.*, 1957, 499.
[10] Wilzbach, *J. Amer. Chem. Soc.*, 1957, **79**, 1013.
[11] Friedmann, Marrian, and Simon-Reuss, *Biochim. Biophys. Acta*, 1952, **8**, 680.
[12] Glascock, " Isotopic Gas Analysis for Biochemists," Academic Press Inc., New York, 1954.
[13] Jaquemin, Michel, Nunez, and Roche, *Compt. rend.*, 1959, **19**, 1904.

alkaline side during reduction: but a more serious objection was the position of the tritium atom in the molecule.

The diphosphate (I; R = R′ = R″ = H) certainly enters the living cell [14] and is fixed there. Dephosphorylation readily occurs [4] with at least partial oxidation to the quinone; the chemical properties of 2-methyl-1,4-naphthaquinone suggest that it will be readily fixed to cell constituents by reaction with thiol groups. This will necessarily replace the atom, be it hydrogen or tritium, occupying position 3, this atom appearing, probably, as part of a water molecule and being dispersed by way of the body pool. Incorporation of tritium into the aromatic ring was therefore to be preferred; the 6-iodo-derivative (I; R = R′ = H, R″ = I) was available [2] as a starting material, but a new method of preparing some of the intermediates has been developed. Catalytic reduction in an alkaline-aqueous dioxan medium yielded 2-methyl-6-tritio-1,4-naphthaquinol bis(disodium phosphate) (I; R = R′ = $^1$H, R″ = $^3$H) of specific activity 27 curies per millimole. Radiochemical purity was confirmed by dilution analysis and by autoradiography of paper-chromatogram strips, the latter indicating the presence of only one radioactive constituent. Oxidation to 2-methyl-6-tritio-1,4-naphthaquinone showed that the tritium was firmly bound even under the strongly acid conditions of ceric sulphate oxidation.[15] Ultraviolet light absorption measurements and paper chromatography showed that dehalogenation was not quite complete and that a small amount of the 6-iodo-compound was still present.

Radiochemical analyses performed after varying periods of time showed that the tritiated compound was relatively stable in aqueous solution at 0°. Solutions exposed to air darkened considerably, but those sealed over nitrogen or in a vacuum remained almost colourless for months.

Although biological and clinical data will be presented fully elsewhere, it may be mentioned that treatment of Ehrlich ascites cells *in vitro* with 2 millicuries per ml. of the monotritiated drug (I; R = R′ = $^1$H, R″ = $^3$H) for 2 hours before inoculation into Swiss white mice had about the same effect on the subsequent tumour growth as a dose of 400—500r *X*-rays *in vitro*. This effect was not shown by a combination of tritiated water and 2-methyl-1,4-naphthaquinol bis(disodium phosphate) (I; R = R′ = R″ = H) at the relevant dose levels.[16] It was also observed [14] that under identical conditions of culture *in vitro*, malignant cells incorporated the radioactive drug much more than did normal cells.

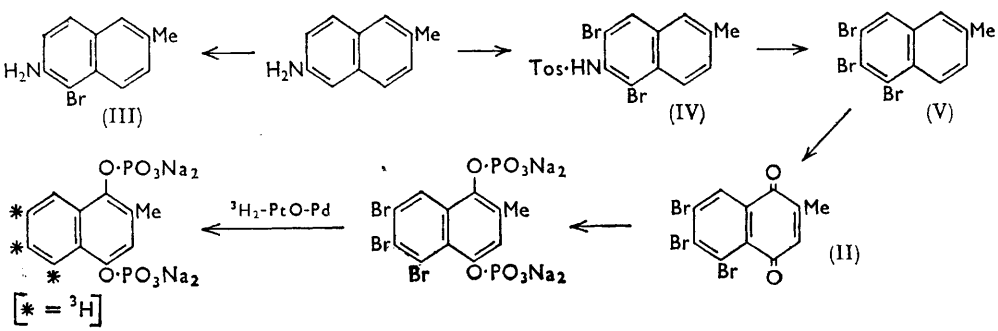
We are using this drug by intra-arterial injection into patients whose malignancies are untreatable by conventional means, and doses of up to 8 curies have been given without measurable effects on the bone marrow; this is consistent with the findings that such treatment gives rise to much greater uptake by the tumour than by normal organs including the marrow, often by a factor of 3 and sometimes 6—7.

Such studies [17] suggested that increasing the specific activity still further by reduction of polyhalogenated quinol bis(disodium phosphates) would be worth while and a route to 2-methyl-5,6,7-tritritio-1,4-naphthaquinol bis(disodium phosphate) (I; R = $^1$H, R′ = R″ = $^3$H) has been completed. Bromination of 6-methyl-2-naphthylamine in acetic acid gave only the monobromo-derivative (III), but treatment of the tosyl derivative in pyridine gave the dibromo-sulphonamide (IV) in good yield, convertible by two routes into 5,6,7-tribromo-2-methylnaphthalene (V) and then, by oxidation, into the quinone (II). Reduction and phosphorylation to the bis(disodium phosphate) (I; R = H, R′ = R″ = Br) gave material readily reducible to the parent compound (I; R = R′ = R″ = H), and, when carrier-free tritium was used, the product contained 3 firmly bound tritium atoms per molecule.

[14] Simon-Reuss, *Acta Radiologica*, 1961, **56**, 49.
[15] Yamagiski, *Ann. Reports Takeda Res. Lab.*, 1954, **13**, 25.
[16] Marrian and Di Vita, unpublished work.
[17] Marrian, Marshall, and Mitchell, unpublished work.

Oxidation of the tribromo-quinone (II) with dilute nitric acid at 150° [18] gave a tribromophthalic acid, showing that all the bromine atoms were in the aromatic ring, probably in the 5,6,7-positions indicated.

In attempts to prepare 5,6,7,8-tetraiodo-2-methyl-1,4-naphthaquinone by a route involving a Diels–Adler condensation of tetraiodobutadiene and toluquinone, only tars or starting materials were recovered.




## Experimental

Ultraviolet light absorption measurements were made on a Unicam S.P. 500 instrument. Tritium was measured by combustion [19] of the samples in oxygen followed by β-scintillation counting of the water produced, by means of either an Ekco Electronics (612) or a Nuclear Enterprises (5503) Counter.

*Irradiation of 2-Methyl-1,4-naphthaquinol Bis(disodium Phosphate) with Tritium.*—The salt (305 mg., containing less than 1% of water of crystallisation) was sealed with tritium (197 c; isotopic purity 98%) and kept at room temperature with occasional shaking for 14 days. Gaseous and volatile products were removed *in vacuo* and the residue [specific activity 35 c/mmole; $\lambda_{max.}$ 296 mμ (ε 4580 in water)] was analysed by dilution. Some of the solid (21·64 mg.) was dissolved in water (50 ml.), and inactive 2-methyl-1,4-naphthaquinol bis(disodium phosphate) (16·987 g.) added. The water was distilled off *in vacuo* and found to contain 2 c of tritium. The dry salt (specific activity 3·7 mc/mmole) was recrystallised from aqueous alcohol and then had a specific activity of 0·4 mc/mmole, unchanged by further recrystallisation. The radiochemical purity of the salt was thus 11% after removal of labile tritium.

*2-Methyl-[³H]-1,4-naphthaquinone.*—A boiling 10% solution of ceric sulphate in 20% sulphuric acid (50 ml.) was filtered into a solution of 2-methyl-[³H]-1,4-naphthaquinol bis(disodium phosphate) (0·5 g.; 0·4 mc/mmole) in water (1—2 ml.). After ~2 minutes' shaking, the pale yellow precipitate was filtered off and washed with water. The quinone was recrystallised from aqueous alcohol, giving 160 mg. (77%) of specific activity 0·4 mc/mmole.

Similar results were obtained by analysis of the product obtained by irradiation of the quinol diphosphate (304 mg.) with tritium (100 c) for 32 days. After removal of labile tritium and recrystallisation, the salt had a specific activity of 0·7 mc/mmole, unchanged by oxidation to the quinone as above; the radiochemical purity was therefore 24%.

*Reduction of 2-Bromo-3-methyl-1,4-naphthaquinol Bis(disodium Phosphate).*—The reaction was carried out in small-scale equipment attached to a high-vacuum line. Hydrogen was transferred by means of a Toepler pump. The hydrated sodium salt (92·4 mg.), 5% palladium–charcoal (10·2 mg.), palladium oxide (1·6 mg.), and water (0·3 ml.) were stirred overnight in the presence of hydrogen (4 ml.). Uptake had then stopped and the ultraviolet absorption of the solution indicated that dehalogenation was essentially complete (ε at 226/235 = 0·67 and 1·53; at 250/260 mμ = 1·38 and 0·55; for the starting material and product, respectively). Repetition of the reaction in the presence of tritium gave a product of minimal specific activity 4·5 c/mmole after removal of labile tritium by dissolution in water and evaporation *in vacuo*.

[18] Tilden and Armstrong, *Brit. Assoc. Adv. Sci., Reports*, 1901, 152; 1902, 176.
[19] Schöniger, *Mikrochim. Acta*, 1955, 123; 1956, 869.

The actual activity may have been considerably higher as, at that time, activity measurements were made on serially diluted samples. It was later shown that, in very dilute solution, the tritiated salt was very strongly absorbed on to glass surfaces, giving rise to large errors in such determinations.

*6-Methylnaphthalene-2-sulphonyl Chloride.*—The following method was more reliable than the published method.[20] Finely powdered sodium 6-methylnaphthalene 2-sulphonate (300 g.) was mixed with redistilled phosphorus oxychloride (600 ml.) in a 2-l. flask which was then heated on a steam-bath with occasional swirling until the contents were mostly liquid (about 80 min.). The mixture was poured over ice in a 5-l. beaker and stirred until the lower layer had solidified (about 45 min.). The beaker was set aside in the refrigerator for 2 hr. with frequent stirring and addition of ice to control the heat which was evolved. The solid was filtered off, washed with water, sucked dry, and ground in a mortar with acetic acid (120 ml.). The almost colourless solid was filtered off, washed with a little methyl alcohol, and dried in air, to give 266 g. (90%) of the chloride, m. p. 90—92° (lit.,[20] m. p. 97—98°), sufficiently pure for reduction to the sulphinate.

*Sodium 6-Methylnaphthalene-2-sulphinate.*—Reduction of the above sulphonyl chloride (200 g.) by zinc dust in aqueous alkali [21] gave 171 g. (91%) of a colourless solid. After 3 recrystallisations from water, the *compound* formed prisms (Found, in material dried *in vacuo* at room temperature: C, 55·9; H, 4·6. $C_{11}H_9NaO_2S,\frac{1}{2}H_2O$ requires C, 56·0; H, 4·2%).

*2-Iodo-6-methylnaphthalene.*—To stirred boiling water (1 l.) was added sodium 6-methylnaphthalene-2-sulphinate (100 g.) and then mercuric chloride (130 g.) together with some hexan-1-ol to reduce frothing. After 2 hours' heating with occasional addition of hexan-1-ol, no more sulphur dioxide was evolved. The solution was cooled somewhat, and the mercurichloride compound was filtered off, washed with water and alcohol, and sucked as dry as possible. The solid was treated under reflux with iodine (66 g.) in alcohol (1 l.). At this point all solids were in solution and a slight excess of iodine was present. The solution was poured into a stirred aqueous solution of potassium iodide and more iodide added until the precipitate was almost colourless. The compound was filtered off, washed with water, and dried at 80° overnight, giving colourless prisms (46 g., 39%), m. p. 145—146° (lit.,[2] 146—147°).

*6-Iodo-2-methyl-1,4-naphthaquinol Bis(disodium Phosphate).*—The method previously described [2] was followed except that lithium hydroxide was used to hydrolyse the phosphorylation residue. When the solution remained at pH 9—10 without further addition, the organic soluble material was extracted with methylene chloride, any precipitate of lithium phosphate filtered off, and acetone (4 vol.) added to the filtrate. An oil settled which crystallised in the refrigerator. The solid was triturated with much alcohol and filtered off. 12 g. of the quinone gave 14·1 g. of the quinol bis(dilithium phosphate) which was not hygroscopic and appeared spectrally to be 80—90% pure [$\lambda_{max.}$ 230 (ε 33,400), 247 (ε 34,100), and 290 mμ (ε 4400) in 0·01N-HCl; lit., ε 34,600, 38,050, and 5740, respectively, at the same wavelengths]. The lithium salt (7·0 g.) was dissolved in water (25 ml.) and converted into the free acid over a column of Dowex 50 ($H^+$). The calculated amount of sodium hydroxide was added and the whole evaporated to dryness *in vacuo* over nitrogen. A solution of the residue in hot methyl alcohol (150 ml.; charcoal) was filtered and cooled. After addition of alcohol to slight opalescence (150 ml.) the whole was gently refluxed and the precipitated solid (reverse solubility) filtered hot and dried *in vacuo* (yield 3·5 g.; colourless prisms). A further 1·0 g. was isolated from the mother-liquors by further addition of alcohol and refluxing, both crops appearing spectrally pure.

*Reductive Dehalogenation of the Salt.*—(*a*) *With hydrogen.* Hydrogenation of the above salt (50 mg.) in water (0·2 ml.) in the presence of platinum oxide (1·5 mg.) and 5% palladium–charcoal (5 mg.) was essentially complete after 4 hr. at room temperature, as determined by change in ultraviolet absorption (ε 250/260 = 5·0 and 0·55 for the starting material and product respectively in 0·01N-HCl) and by paper chromatography. (*b*) *With tritium.* The sodium salt (55 mg.) was dissolved in 0·5N-sodium hydroxide (0·2 ml.) and dioxan (0·4 ml.). Platinum oxide (2 mg.) and 5% palladium–charcoal (5 mg.) were added and the mixture stirred for 16 hr. with tritium (2 ml.; 5 c). The reaction was completed by stirring for a further hour with hydrogen. Total uptake was 2·65 ml. at 20°. After removal of labile tritium, dilution analysis demonstrated that the product, which contained 27 c/mmole, had radiochemical purity of 100%.

[20] Bendich and Chargaff, *J. Amer. Chem. Soc.*, 1943, **65**, 1568.
[21] Cf. *Org. Synth.*, 1951, Coll. Vol. I, 492.

*Stability of 2-Methyl-6-tritio-1,4-naphthaquinol Bis(disodium Phosphate).*—By the methods indicated above, the following results were obtained:

| Specific activity c/mmole | Storage conditions and temp. | | Age (months) | Radiochem. purity (%) |
|---|---|---|---|---|
| 0·4 | Solid | 18° | 13 | 75 |
| 0·4 | Solution | 18 | 13 | 87 |
| 19 | ,, | 0 | 2 | 88 |
| 27 | ,, | 0 | 2 | 100 |
| 27 | ,, | 0 | 3·5 | >90 |

*1-Bromo-6-methyl-2-naphthylamine Hydrochloride.*—6-Methyl-2-naphthylamine [22] hydrochloride (1·35 g.) was dissolved in acetic acid (50 ml.) and cooled to incipient crystallisation. A stream of air was passed through bromine and into the amine solution with shaking. When an excess of bromine was clearly present, the precipitated solid was filtered off and washed with light petroleum. The *product* was purified by dissolving it in 50% aqueous alcohol and adding concentrated hydrochloric acid (20 ml.) [yield, 1·2 g., 73%; m. p. 215° (decomp.) after sintering at 212°]. Further recrystallisations from the same mixture gave the amine hydrochloride as colourless needles, m. p. 219° (decomp.) (Found, in material dried *in vacuo* at room temperature: C, 48·9; H, 4·2; N, 5·1. $C_{11}H_{11}BrClN$ requires C, 48·5; H, 4·1; N, 5·1%).

*N-(1,3-Dibromo-6-methyl-2-naphthyl)toluene-p-sulphonamide.*—6-Methyl-2-naphthylamine hydrochloride (100 g.) in dry pyridine (500 ml.) was treated with toluene-*p*-sulphonyl chloride (100 g.) in dry pyridine (250 ml.) with stirring. The deep red solution was heated on the steam-bath for 1 hr., allowed to cool to room temperature, and treated dropwise, with stirring, with a solution of bromine (56 ml.) in acetic acid (150 ml.). The maximum temperature during the addition was 50°. When cool, the mixture was stirred vigorously during the addition of water (1 l.), and the granular solid filtered off, washed with water, suspended in a blender with methyl alcohol, and recovered by filtration and drying. The crude sandy solid (198 g., 81%) had m. p. 194—196° and was sufficiently pure for deacylation. For analysis, the *compound* was recrystallised from 2-ethoxyethanol to m. p. 203° (Found, in material dried at room temperature: C, 46·2; H, 3·5; N, 3·3. $C_{18}H_{15}Br_2NO_2S$ requires C, 46·1; H, 3·2; N, 3·0%).

*1,3-Dibromo-6-methyl-2-naphthylamine.*—The above crude tosyl derivative (5 g.) was dissolved in concentrated sulphuric acid (50 ml.) with shaking and kept at room temperature for 45 min. The solution was poured on crushed ice, and the solid filtered off, washed with water, suspended twice in N-sodium hydroxide, washed free from alkali with water and finally with methyl alcohol. Recrystallisation from 2-ethoxyethanol gave the *amine* (2 g., 60%) as colourless prisms, m. p. 131°. A further 1 g. (m. p. 128°) was isolated from the hot mother-liquors by dilution with water. Recrystallisation of the first crop from alcohol, butan-1-ol, or light petroleum did not raise the m. p. (Found, in material dried *in vacuo* at room temperature: C, 42·1; H, 3·0; N, 4·5. $C_{11}H_9Br_2N$ requires C, 41·9; H, 2·9; N, 4·45%).

*1,2,3-Tribromo-6-methylnaphthalene.*—(*a*) Concentrated sulphuric acid (148 ml.) was cooled to 5° and stirred during the addition of finely powdered sodium nitrite (21·2 g.).[23] The mixture was then heated to 70° until dissolution was complete, cooled to 10°, and stirred during the gradual addition of a slurry of 1,3-dibromo-6-methyl-2-naphthylamine (88 g.) in acetic acid (1 l.) at <20°. The yellow solution was then added to a solution of cuprous bromide (35·5 g.) in concentrated hydrobromic acid (590 ml.). The initially purple solution became brown, gas was evolved, and a precipitate formed with return of the purple colour. When gas evolution had ceased, water (2 l.) was added, and the solid filtered off and washed with water and then with methyl alcohol. After recrystallisation from ethylene glycol, there were obtained 54 g. (50%) of colourless needles, m. p. 105—108°. A further 12 g. (11%), m. p. 102—104°, were obtained from the mother-liquors. For analysis, the *compound* was recrystallised twice more, to m. p. 109° (Found, in material dried *in vacuo* at 50°: C, 35·0; H, 2·1. $C_{11}H_7Br_3$ requires C, 34·9; H, 1·9%).

(*b*) 1,3-Dibromo-6-methyl-2-naphthylamine (1·4 g.) was dissolved in warm acetic acid (20 ml.), cooled below 20° with stirring, and treated with sodium nitrite (2 g.) in concentrated sulphuric acid (14 ml.) prepared as above. After 10 min., the mixture was treated dropwise with bromine (2 ml.) in a 50% solution of hydrobromic acid in acetic acid (20 ml.) until no further

[22] Dziewonski, Schoenowna, and Waldmann, *Ber.*, 1925, **58**, 1216.
[23] Hodgson and Walker, *J.*, 1933, 1620.

precipitate was formed.[24] The solid diazoperbromide was filtered off, then refluxed for 20 min. with chloroform (20 ml.), and the insoluble material refluxed with 1 : 1 v/v acetic anhydride–acetic acid (25 ml.) until dissolution was complete. On cooling, the product formed light brown needles (1·2 g., 71%), m. p. 102—103° which, after recrystallisation, did not depress the m. p. of a sample prepared by method (*a*).

5,6,7-*Tribromo*-2-*methyl*-1,4-*naphthaquinone*.—1,2,3-Tribromo-6-methylnaphthalene (5 g.) was stirred in acetic acid (100 ml.) during the dropwise addition of a solution of chromium trioxide (6·6 g.) in water (6·6 ml.) and acetic acid (10 ml.) at <40°. The mixture was stirred and warmed to 80° and, after 35 min., cooled to room temperature. The *tribromo-quinone* (1·2 g.; m. p. 188°) was filtered off and washed with water. Recrystallisation from pentyl acetate or 2-ethoxyethanol gave orange needles (0·8 g.), m. p. 194—196° [Found, in material dried *in vacuo* at room temperature: C, 32·5; H, 1·6; Br, 58·8. $C_{11}H_5Br_3O_2$ requires C, 32·3; H, 1·2; Br, 58·6%), $\lambda_{max.}$ 266 (ε 23,200), $\lambda_{min.}$ 230 mμ (ε 10,600)].

Dilution of the original acetic acid mother-liquors gave a *compound* which recrystallised from pentyl acetate in bright yellow needles (0·2 g.), m. p. 193—195°. A further recrystallisation raised the m. p. to 195° (Found, in material dried *in vacuo* at room temperature: C, 40·25; H, 2·2; Br, 47·5. $C_{11}H_6Br_2O_2$ requires C, 40·0; H, 1·8; Br, 48·4%), $\lambda_{max.}$ 253 (ε 15,620), 260 (ε 14,900) and 294—295 mμ (ε 13,300), $\lambda_{min.}$ 257 (ε 13,900) and 270 mμ (ε 4,640).

When heated overnight in a sealed tube at 150° with dilute nitric acid (*d* 1·15),[19] the tribromo-quinone furnished colourless prisms, m. p. 199—201° (Found: Br, 60·3%; equiv., 197. $C_8H_3Br_3O_4$ requires Br, 59·5%; equiv. 201·5). A tribromophthalic acid, of unknown orientation,[25] is reported as having m. p. 190—191°.

5,6,7-*Tribromo*-2-*methyl*-1,4-*naphthaquinol Bis*(*disodium Phosphate*).—Reduction and phosphorylation of 5,6,7-tribromo-2-methyl-1,4-naphthaquinol (4 g.) by the procedures described above for the 6-iodo-quinone yielded the *tetrasodium salt* (4·5 g.) as colourless prisms which slowly became pink (Found, in material dried *in vacuo* at room temperature; C, 17·5; H, 2·6; Br, 30·8; P, 8·5; $H_2O$, 18·0. $C_{11}H_5Br_3Na_4O_8P_2,7H_2O$ requires C, 16·8; H, 2·4; Br, 30·5; P, 7·9; $H_2O$, 16·1%), $\lambda_{max.}$ 252 (ε 57,200), 250 (ε 56,500), and 256 mμ (ε 55,400) in $H_2O$, 0·01N-HCl, and 0·5N-NaOH, respectively.

*Hydrogenation of* 5,6,7-*Tribromo*-2-*methyl*-1,4-*naphthaquinol Bis*(*disodium Phosphate*): *Reductive Dehalogenation*.—(*a*) *With hydrogen*. Under the same conditions as for the 6-iodo-compound, reduction was essentially complete overnight as shown by ultraviolet spectrum (ε 250/290 changing from 8·8 to 0·3) and by paper chromatography in saturated ammonium sulphate–M-sodium acetate–isopropyl alcohol (80 : 20 : 2). (*b*) *With tritium*. The sodium salt (90 mg.) was dissolved in 3N-sodium hydroxide (0·3 ml.), and platinum oxide (2 mg.) and 10% palladium–calcium carbonate (25 mg.) were added. The mixture was stirred for 5 hr. with tritium gas (10 ml.; 25 c), the total uptake being 7 ml. at 20°. Isolation of the product as previously described yielded 2-*methyl*-5,6,7-*tritritio*-1,4-*naphthaquinol bis*(*disodium phosphate*) having a total activity of 9 c (83 c/mmole). Dilution analysis indicated a radiochemical purity of 100%, confirmed by chromatography. The ultraviolet absorption spectrum was identical with that of the parent compound.

The authors thank Dr. A. L. Morrison, Roche Products, Welwyn Garden City, for gifts of "Synkavit" [2-methyl-1,4-naphthaquinol bis(disodium phosphate)] and other compounds, Mr. W. C. Spurden for technical assistance, and Dr. E. J. Wilson, Isotope Division, A.E.R.E., Harwell, for advice and help with the Wilzbach irradiation experiments. A grant from the International Atomic Energy Agency in aid of this work is gratefully acknowledged. One of us (M. G.) is in receipt of a Medical Research Council training grant.

Department of Radiotherapeutics, University of Cambridge.
Radiochemical Centre, Amersham.
Roche Products, Welwyn Garden City. [*Received, March 2nd,* 1962.]

[24] Konsden and Kenyon, *J.*, 1935, 1591.
[25] Flessa, *Ber.*, 1884, **17**, 1482.