SYNTHESIS OF 2,3-^{125}DIIODO-5-t-BUTYL-1,4-BENZOQUINONE

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Received February 20, 1978

SUMMARY

2,3-Diiodo-5-t-butyl-1,4-benzoquinone, an inhibitor of photosynthetic electron transport, was prepared ^{125}iodine-labelled for studies of its mechanism of action. The synthesis was carried out by reaction of 2,3-dibromo-5-t-butyl-1,4-benzoquinone with Na^{125}J.

Key Words: photosynthesis, inhibitor, quinone, ^{125}iodine

INTRODUCTION

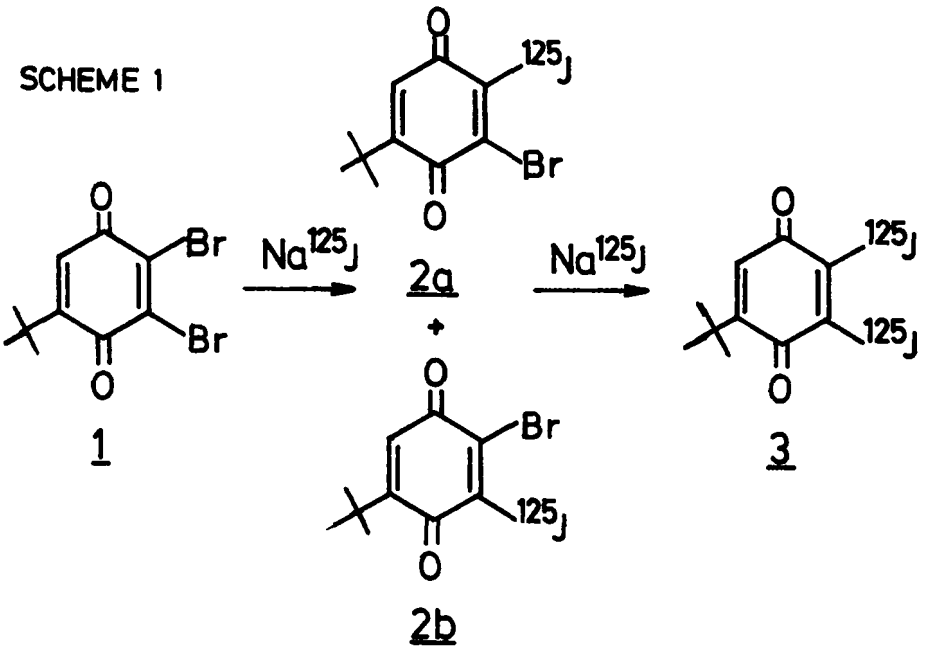
2,5-Dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (DBMIB) has been introduced by Trebst et al. (1) as a powerful inhibitor of photosynthetic electron transport. It allowed investigations on electron transport and photophosphorylation connected either to photosystem I or photosystem II (2). For detailed studies of its mechanism of action, a synthesis of a radioactive DBMIB was desirable. A synthesis of a ^{14}C-labelled DBMIB would have included several steps and the starting material turned out to be rather expensive.

In course of our investigations on structure-activity relationship of halogenated 1,4-benzoquinones, we had also synthesized 2,3-diiodo-5-t-butyl-1,4-benzoquinone (3). Its inhibitory activity (pI_{50} = 7.5) and its site of inhibition are virtually identical to DBMIB (3,4). Its synthesis from 2,3-dibromo-5-

0362-4803/78/0015-0581$01.00

t-butyl-1,4-benzoquinone (1) by reaction with NaJ opened a convenient, one-step synthetic route to a labelled inhibitor of the DBMIB type (Scheme 1).

SCHEME 1



The preparation, however, was complicated by the fact that the starting material 1, the intermediates 2a and 2b, and the reaction product 3 are not separated by column or thin layer chromatography, and are separable only by gas chromatography. This difficulty can be overcome by applying an excess of NaJ, which will shift the equilibrium of the exchange reaction to the right side in Scheme 1. This in consequence will lead to a lower radiochemical yield of 3. The reaction kinetics at an excess of 100 % NaJ can be seen in Fig. 1. Under these conditions, the yield of 3 is almost quantitative, there are only traces of 2a and 2b and of an unknown impurity present, also no starting material 1 is detectable.

In the radioactive synthesis, the overall radiochemical yield

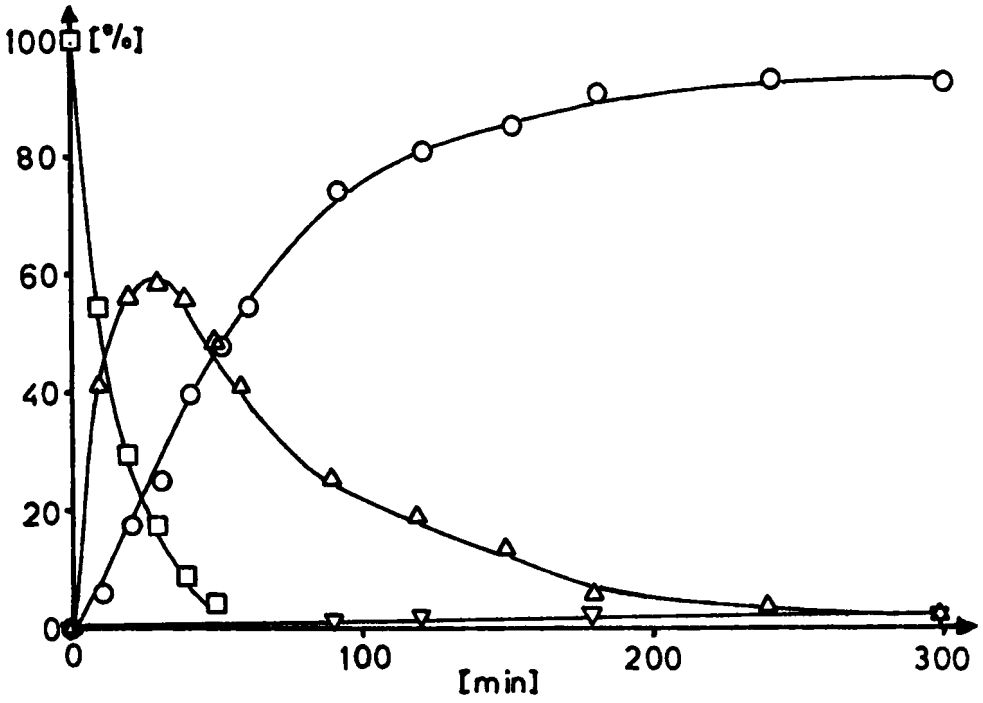


Fig. 1. Kinetics of the reaction in Scheme 1 as followed gas-chromatographically. □---□ 1, Δ---Δ 2a and 2b, O---O 3, and ▽---▽ an unknown impurity.

was 49 % based on Na^{125}J at a specific activity of 49 mCi/mMol. Labelled and unlabelled compound showed no difference in chemical and biochemical behaviour.

EXPERIMENTAL

NMR spectra were recorded on a Perkin-Elmer R 12 A with TMS as internal standard and optical spectra on a Cary 15. For gas-chromatography, a Varian Aerograph, series 1400 (column 5 ft, 1/4', 5 % silicon SE-30 on Chromosorb WAW DMCS 60/80, oven 170 °), connected to an integrator, model 477, was used. The melting points are not corrected.

2,3-Dibromo-5-t-butyl-1,4-benzoquinone (1). 16.4 g (0.1 Mol) of 2-t-butyl-1,4-benzoquinone and 17 ml of bromine in 300 ml acetic acid are kept at 80 °C for 2 hrs. Then air is bubbled through the reaction mixture to remove excess bromine and the hydrogen bromide formed. The mixture is poured into 3 l of water and the precipitate filtered with suction. Yield 28.0 g (87 %). Recrystallized from ethanol; mp. 92 °C (ref. (5) 92-93 °C).

2,3-Diiodo-5-t-butyl-1,4-benzoquinone (3). 9.66 g (0.03 Mol) of 1 and 27.00 g (0.18 Mol) of finely powdered NaJ in 200 ml diethylketone are stirred for 4 hrs at 100 °C. The solvent is evaporated in the vacuum, to the residue 200 ml of water are added and the mixture is extracted several times with ether. After drying with $MgSO_4$, the ether is evaporated in the vacuum. Yield 11.8 g (96 %). Recrystallized from 80 % ethanol; mp. 102 °C.

$C_{10}H_{10}J_2O_2$ (416.0) Calc. % C 28.87 H 2.42 J 61.01

Found % C 28.97 H 2.40 J 60.94

Absorption spectrum (methanol): 282 nm (ε = 10 250 $mM^{-1}cm^{-1}$), 402 nm (ε = 1 450 $mM^{-1}cm^{-1}$).

NMR (δ, CCl_4): singlet 1.30 (9 protons); singlet 6.86 (1 proton).

2,3-^{125}Diiodo-5-t-butyl-1,4-benzoquinone (3). 20 mCi $Na^{125}J$ with carrier 0.1-1 mg NaJ in 0.5 ml water (Amersham Buchler GmbH & Co KG, D-3000 Braunschweig, W.-Germany) are diluted with 120 mg NaJ (0.8 mMol) in 2 ml of water, evaporated to dryness in the vacuum and dried for 24 hrs over P_2O_5. 66 mg (0.2 mMol) of 1 in 5 ml diethylketone are added and the mixture stirred at 100 °C for 5 hrs. Gaschromatographic analysis showed a purity of 98 % for the reaction product 3. For biochemical experiments, small samples of about 70 µl are withdrawn from the stock solution and chromatographed on silica gel pre-coated plastic sheets (Polygram SIL G/UV_{254}, thickness 0.25 mm; Macherey-Nagel+Co GmbH, D-5160 Düren, W.-Germany) with benzene as the solvent. The zone, cor-

responding to 3 (R_f = 0.51) is cut out and eluted with methanol. The concentration of 3 is determined from its absorption at 282 nm.

ACKNOWLEDGMENT

This work was supported by Deutsche Forschungsgemeinschaft. I am indebted to Mr. K. Masson for skilful technical assistance.

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