Studies on Mustard Gas ($\beta\beta'$ -Dichlorodiethyl Sulphide) and some Related Compounds

3. THE PREPARATION AND USE OF MUSTARD GAS CONTAINING (a) RADIOACTIVE SULPHUR AND (b) DEUTERIUM†

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For many of our investigations it was our intention to use mustard gas containing either radioactive sulphur (S^{35}) or deuterium, or if possible both of these isotopes, as tracer elements. The first two compounds, 'radio'-mustard gas (H^*) and 'deutero'-mustard gas, were prepared as described below, and the former was used in many of the investigations described in this series of papers.

EXPERIMENTAL

PREPARATION OF MUSTARD GAS (H), AND RELATED COMPOUNDS, CONTAINING RADIOACTIVE SULPHUR (S^{35})

In all experiments where a compound containing S is used, an asterisk (e.g. S^* , H^* , HO^* , HO_2^*) denotes the radio-compound as distinct from the ordinary material.

The S^* with which this work was carried out was prepared and separated as $BaSO_4^*$ as described by Banks (1946), or was obtained (as $BaSO_4^*$) through the Ministry of Supply from the U.S.A.

Mustard gas (H^) containing radioactive sulphur*

(i) *Using sulphur chloride.* The $BaSO_4^*$ was reduced to BaS^* by heating it in a muffle furnace with twice its weight of sugar carbon in a graphite crucible (coated with alundum cement to minimize burning) for 10 hr. at about 900° . The resulting mixture was heated in a tube-furnace in a current of pure dry Cl_2 , and the product, mainly sulphur mono- and dichlorides, was condensed in a glass spiral at -20° . Ethylene was then passed into this mixture (warmed to 60°), and the crude H^* distilled at $114^\circ/20$ mm.

This method was later abandoned because of the unavoidable loss of S^* as a by-product of the reaction between ethylene and S_2Cl_2 . This S^* was normally reoxidized to $BaSO_4^*$ for further use, together with any residue in the distillation flask.

(ii) *Via thiodiglycol** (cf. Othmer & Kern, 1940; Kistiakowsky, 1940). The $BaSO_4^*$ was reduced to BaS^* in a tube-furnace in a stream of dry hydrogen at 1000° for 4 hr., any H_2S^* formed being trapped in alkaline zinc chloride solution. The H_2S^* was liberated by treating the BaS^* , plus trap contents, with a little granulated zinc and 50 %

phosphoric acid, passed through a reflux condenser and P_2O_5 trap to remove water vapour, and finally frozen out in an all-glass apparatus by means of liquid air. The stream of H_2 generated by the action of the acid on the zinc ensured that no H_2S^* remained in the generator or the drying tube, and removal of the last traces was hastened by boiling the generator for some time towards the end of the reaction.

The apparatus was then evacuated and the H_2S^* was allowed to evaporate into a glass reaction vessel of known volume, with a side-arm closed off by a tap, and a barometer tube attached for measuring the pressure. The H_2S^* (81 % yield based on $BaSO_4^*$) was then recondensed in the side limb, and air-free ethylene oxide was evaporated into the reaction vessel to twice the H_2S^* pressure. On re-evaporation of the H_2S^* , the reaction proceeded at room temperature, with a quantitative yield of thiodiglycol* (TG^*) within 48 hr. The quantity of $BaSO_4^*$ used in each preparation (about 3 g.) was calculated to yield an H_2S^* pressure of about half an atmosphere in the reaction vessel.

The TG^* , dissolved in dry chloroform, was converted to H^* by treatment with a slight excess of thionyl chloride in chloroform solution at $50-60^\circ$. (Overall yield of H^* : 74 %.)

H-sulphoxide (HO^*); $\beta\beta'$ -dichlorodiethyl sulphoxide*

This was prepared from 154 mg. of H^* by the method of Helfrich & Reid (1920); it was recrystallized from 0.3 ml. of water, and dried *in vacuo* over P_2O_5 ; yield 74 mg. HO^* , m.p. 109° .

H-sulphone (HO_2^*); $\beta\beta'$ -dichlorodiethyl sulphone*

109 mg. of H^* were oxidized to HO^* , and the crude crystalline product remaining after removal of the HNO_3 by repeated addition and evaporation of water *in vacuo*, was further oxidized to HO_2^* with CrO_3 in presence of 12 % H_2SO_4 (Helfrich & Reid, 1920). The HO_2^* was finally recrystallized from alcohol. (Yield 37 mg., m.p. 55° .)

DETERMINATION OF S^* IN RADIOACTIVE MATERIALS

Oxidation of S^ -containing materials to $BaSO_4^*$*

The technique adopted was a modification of the method of Pirie (1932) for the determination of sulphur in urine. The material to be oxidized (corresponding to not more than 50 mg. and preferably about 20 mg. of $BaSO_4^*$) was treated in a 6×1 in. Monax test-tube with 5 ml. of conc. HNO_3 , 2.5 ml. of 10 % $NaClO_3$ and about 50 mg. of copper wire, and

† Most of the work described in this paper was incorporated in reports to the Ministry of Supply in 1940-3 and the Medical Research Council in 1940.

heated in an air-bath at 200–280°. The treatment with HNO_3 and NaClO_3 was repeated until the resulting dry material was black or dark green, and redissolved to give a clear blue-green solution. At the completion of the incineration, the residue was dissolved in a few drops of conc. HCl , and the sulphate* was precipitated as BaSO_4^* from the boiling diluted solution; the precipitate was collected on a Whatman no. 42 paper, ignited and weighed.

The accuracy of the method was tested by determinations carried out on H , HO , HO_2 and ' HO_2 -tyrosine' (cf. Bourns-nell, Francis & Wormall, 1946*a*), 100% recovery being obtained in each case. In order to observe whether the presence of other substances would interfere, determinations were carried out on H in the presence of water, triolein, and gelatin; in these cases also, the method was found to be quite satisfactory. It was found, however, that H which had first been hydrolyzed by alkali gave variable and low results (often about 80%), due to loss of volatile S-containing substances (possibly divinyl sulphide) produced during the hydrolysis.

Determination of radioactivity

All the radioactivity measurements were made with a side-window Geiger-Müller counter with a thin mica or aluminium window as described by Banks (1946). Suitable BaSO_4^* samples (about 20 mg.) were ground to a paste with two or three drops of water in an agate mortar, and suspended in about 15 ml. of water. This was poured into a small Buchner funnel in which water was being gently drawn through a 4.25 cm. Whatman no. 42 paper, and even spreading was obtained so long as the paper was not allowed to become dry until all the BaSO_4^* had been washed into the funnel.

The amount of S^* in each sample was determined by comparing its count with that, recorded on the same counter and on the same day, given by a standard specimen of BaSO_4^* prepared from a known weight of the H^* , HO^* or HO_2^* used for that experiment. Where more than 20 mg. BaSO_4^* were used, a correction was made for 'self-absorption', i.e. absorption of the soft β -radiation by the relatively thick layer of BaSO_4^* , as described by Banks (1946).

The effect of the radioactivity of S^{35} on living tissues

In experiments to be reported in subsequent papers (Bourns-nell, Cohen, Dixon, Francis, Greville, Needham & Wormall, 1946; Bourns-nell, Francis & Wormall, 1946*b*), H^* and some of its derivatives have been injected into rabbits and the S^* contents of various tissues have been determined.

The maximum concentration of S^* in any of these tissues was found to be 9.25 $\mu\text{g./g.}$ wet tissue (kidney, 1 hr. after injection of H^*). With the specific activity of the H^* used in this particular experiment this corresponds to about 1.2×10^{-9} curie, or 4.4×10^{-8} röntgen/sec. In considerations of protection from radiation, a constant intensity of 10^{-6} röntgen/sec. is considered safe over a long period of time (1 year or more) (Recommendations of the British X-Ray and Radium Protection Committee, 1938). The maximum intensity achieved in our animal experiments is thus about 1/200th of this figure. (We are indebted to Dr T. E. Banks for these calculations.)

PREPARATION OF MUSTARD GAS CONTAINING DEUTERIUM

This was carried out, following the method of Gibson & Pope (1920), by the repeated passage of deuterio-ethylene through freshly prepared sulphur monochloride.

Sulphur monochloride. Dry Cl_2 was passed over fused roll sulphur in a retort, and the crude product was fractionally distilled with the addition each time of a small quantity of powdered roll sulphur. B.p. 135°.

Deuterium from D_2O . Deuterium was prepared by electrolysis of a 6% solution of KOH in 99.65% D_2O in a 25 ml. U-shaped cell fitted with platinum electrodes. A quantity of glass beads, included to minimize any mixing of oxygen and deuterium and to reduce the 'dead space' in the cell, was found not greatly to reduce the conductivity of the cell.

Deutero-ethylene. A mixture of equal volumes of deuterium and deutero-acetylene (prepared by the action of D_2O on calcium carbide) was passed at about 6 l./hr. through a combustion tube containing a palladium-kieselguhr catalyst (1:900) supported on asbestos wool and maintained at 150° (Yoshikawa, 1932). The catalyst was made by the method of Willstätter & Waldschmidt-Leitz (1921). All the gases were kept over saturated brine to reduce loss by solution in the water.

A trace of acetylene in the product was removed by ammoniacal Cu_2Cl_2 ; propylene was removed in a CO_2 -ethanol trap at -65° , and the crude ethylene was fractionally distilled at -110 to -105° , to separate from ethane. The purity of the final product was checked by determinations of vapour density and degree of unsaturation. This latter determination was carried out by treating a known volume of gas (confined over brine in a Hempel burette) with a measured volume of N-KBrO_3 and excess of 10% KBr and 3*N*- HCl . The total percentage of unsaturated gases was calculated from the contraction in volume, and the degree of unsaturation of this fraction by determining the excess of bromine, by adding KI and titrating with standard thiosulphate. The finally purified gas contained 90% deutero-ethylene and 10% saturated 'deutero-hydrocarbons'.

Combination of deutero-ethylene with sulphur monochloride

The reaction was effected by heating the sulphur chloride to 60° and passing a slight excess of the gas through it by means of a pump mechanism, in which, by reversing a pair of two-way taps, the deutero-ethylene flowing to and from one aspirator bottle to another was passed repeatedly in the same direction through the reaction vessel.

At the end of the reaction, the product was heated to 100° for 1 min., cooled, and the crude H poured off from the semi-solid deposit of sulphur. After one distillation (b.p. 112–119°/20 mm.) 10 g. of deutero-mustard gas was obtained (from 6.1 l. of the deutero-ethylene containing 5.5 l. of the pure gas, i.e. a 51% yield). No determination has yet been made of the amount of deuterium in this 'deutero'- H , but the ratio of deuterium to hydrogen atoms in the compound is probably at least 99:1. No difference in boiling-point at about 20 mm. was detectable between the 'deutero'- H and ordinary H , but the thrice-distilled 'deutero'- H had m.p. 15.7° compared with 14.4° for ordinary H .

Skin tests on human subjects showed no significant difference between the vesicant power of 'deutero-*H*' and that of ordinary *H*.

SUMMARY

1. The preparation, by two methods, of mustard gas containing radioactive sulphur (S^{35}) is described.

2. Methods are described by which the radio-sulphur in S^{35} -containing mustard gas and related compounds, and in biological materials treated with these substances, can be determined.

3. The possible effect on living tissue of the radiation from the S^{35} -containing compounds used in these investigations is briefly discussed.

4. A synthesis of mustard gas with practically all its hydrogen replaced by deuterium is described.

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Studies on Mustard Gas ($\beta\beta'$ -Dichlorodiethyl Sulphide) and some Related Compounds

4. THEIR ACTION ON PROTEINS (STUDIED WITH THE AID OF RADIOACTIVE SULPHUR)†

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Treatment of serum proteins with mustard gas (*H*), under physiological conditions of pH and temperature, effects a very marked alteration in their immunological properties (Berenblum & Wormald, 1939). There is also evidence that several other proteins undergo distinct changes, including an increase in the S content, as a result of the action of *H*; nucleoprotein, for example, is precipitated (Berenblum, 1940); keratine reacts with *H*, in part at least by virtue of its SH groups (Peters & Wakelin, 1941) and collagen is also attacked (Pirie, 1941).

These observations suggest a definite combination of *H* with groups which are present in a variety of

proteins, and since the reaction takes place fairly rapidly at 37° and at pH 7.5, it is not improbable that similar combination occurs when *H* comes into contact with the proteins of the skin and neighbouring tissues [Berenblum (1940), for example, noted that no nucleoprotein could be extracted from *H*-treated rabbit skin]. It was decided, therefore, that a further investigation of this reaction should be made with the aid of H^* , i.e. mustard gas containing radioactive sulphur. (In this paper an asterisk is used to denote the presence of the radioactive S^{35} in a substance, e.g. H^* , S^* , TG^* , HO^* etc.)

This method has the advantage that the amount of S^* present can readily be determined, however small it is, and, within certain limits, however much

† The investigations described here were the subject of reports to the Medical Research Council in 1940-2 and the Ministry of Supply 1941-5.