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

2. THE ACTION OF MUSTARD GAS, $\beta\beta'$-DICHLORODIETHYL SULPHONE AND DIVINYL SULPHONE ON AMINO-ACIDS†

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It is clearly established that mustard gas (H) and the corresponding sulphone (HO_2) react with serum proteins to produce protein derivatives which have immunological properties different from those of the untreated proteins (Berenblum & Wormall, 1939). There is also evidence that certain glycolytic processes are inhibited by H and HO_2 (Berenblum, Kendal & Orr, 1936), and that HO_2 inhibits brain-tissue respiration (Peters, 1936); it is not unlikely that these inhibitory effects are due to the action of H and HO_2 on enzymes or on other proteins in the tissues concerned.

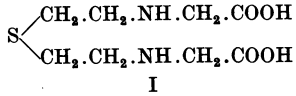
In view of the marked vesicant action of H and its anticarcinogenic action (Berenblum, 1929), it would be of interest to know which groups of the protein molecule react with H, and as part of an investigation of this problem, experiments have been carried out to determine whether or not H and related compounds (HO_2 and divinyl sulphide) react with amino-acids under physiological conditions of pH and temperature. Also, it was thought that any H- and HO_2-derivatives of amino-acids obtained in these experiments would be of considerable value in the further studies which were being made of the immunological properties of H- and HO_2-treated proteins. As far as possible, these experiments with amino-acids were carried out at pH 7·5–8·5 and at 30–40°; in a few special cases, however, more drastic treatment was applied, similar to that employed by Cashmore & McCombie (1923) and Lawson & Reid (1925) for the preparation of HO_2-derivatives of glycine and phenylalanine, viz. in boiling aqueous or ethanol solution in the presence of strong alkali.

A study has also been made of the action of divinyl sulphone on amino-acids, to run parallel with the similar investigations on proteins, and a comparison has been made of the relative rates of reaction of HO_2 and divinyl sulphone on the amino-groups. This last-named investigation is of particular interest in view of the possibility that HO_2 might undergo conversion into divinyl sulphone before reacting with amino- and other groups of amino-acids and proteins.

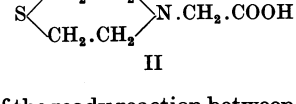
† The work described in this paper was the subject of reports to the Medical Research Council in 1940, and the Ministry of Supply in 1940-2.

The action of mustard gas (H) on amino-acids and their esters

Cashmore & McCombie (1923) have reported the preparation of an open-chain compound, diglycinodiethyl sulphide (I), by the action of H on glycine ester hydrochloride in boiling ethanol solution in the presence of Na_2CO_3 and Na acetate. All our attempts to obtain this compound have failed, but instead, a new product has been isolated and shown to have the structure of *1:4-thiazan-4-acetic acid* (II). The evidence for a structure of this type is based on (*a*) analysis of the free acid, its picrate and sulphilimine, and the picrate and platinichloride of the ethyl ester, and (*b*) titration with NaOH in ethanol solution. The free acid tenaciously retains a molecule of water, and the possibility of the compound having an open-chain structure (β-hydroxy-β'-glycinodiethyl sulphide) had to be considered; it was, however, rejected because no evidence of the presence of an NH or an alcoholic group could be obtained:



I



II

In view of the ready reaction between HO_2 and the amino-groups of amino-acids, we have attempted to prepare H-amino-acid derivatives under physiological conditions of pH and temperature, but without success (cf. however, Bergmann, 1942). Similarly, no reaction could be induced, under these conditions, between divinyl sulphide and amino-acids. Evidence was obtained, however, that H rapidly reacts with the SH group of cysteine and more slowly with arginine, the latter reaction being accompanied by a reduction in amino-N (formol titration).

Many other efforts have been made to obtain H-derivatives of glycine and alanine by heating the amino-acids with H and varying amounts of Na_2CO_3 and Na acetate (to neutralize any HCl liberated), but

all were unsuccessful. An experiment carried out by the Cashmore & McCombie method, but using alanine ester hydrochloride instead of the glycine compound, did not yield an '*H*-alanine' compound analogous to the glycine compound (II); some reaction between the *H* and the amino-group of alanine appeared to have taken place, but in view of the very drastic conditions of this method of preparation, and the improbability that the reaction would resemble those taking place in the animal body, no further investigation of the product was made.

The action of ββ′-dichlorodiethyl sulphone (HO_2) and divinyl sulphone on amino-acids

Using different methods, but both involving reactions at high temperature (in boiling aqueous or ethanol solutions) and in the presence of an excess of alkali, Cashmore & McCombie (1923) have prepared 'HO_2-glycine' (1:4-sulphonazan-4-acetic acid) and Lawson & Reid (1925) 'HO_2-glycine' and 'HO_2-phenylalanine' (1:4-sulphonazan-4-α-benzylacetic acid).

In our experiments, running parallel with those on the preparation of HO_2-proteins (Banks, Boursnell, Francis, Hopwood & Wormall, 1946), we have prepared the HO_2-derivatives of several amino-acids by reactions carried out under physiological conditions of pH and temperature. Solutions of amino-acids in $NaHCO_3$ solution (pH 8–8·5) were treated with ββ′-dichlorodiethyl sulphone (HO_2) and the mixtures stirred at temperatures between 20° and 40° for a few hours. Equimolecular quantities of HO_2 and amino-acid were usually used, and the yields were appreciably better than those obtained by the more drastic methods mentioned above. Since the completion of this work, we have learned that Ford-Moore & Lidstone (1940) have employed a similar technique for the preparation of HO_2-amino-acid derivatives.

An experiment with cysteine showed that HO_2 reacts even more quickly with the SH group than does *H*, and the possibility of a reaction of this type must be kept in mind in connexion with the interaction of HO_2 and SH-containing proteins.

Since divinyl sulphone reacts with proteins to give products which react immunologically and in other respects exactly like the corresponding HO_2-proteins, the action of the former sulphone on glycine has been studied. The product was identical with 'HO_2-glycine'.

Comparison of the rate of action of HO_2 with that of divinyl sulphone on the amino-groups of glycine and alanine

Serological experiments having shown that divinyl sulphone reacts much more rapidly than does HO_2 on serum proteins to produce protein derivatives which will give precipitates with an antiserum to HO_2-proteins (Boursnell, Francis & Wormall, 1946),

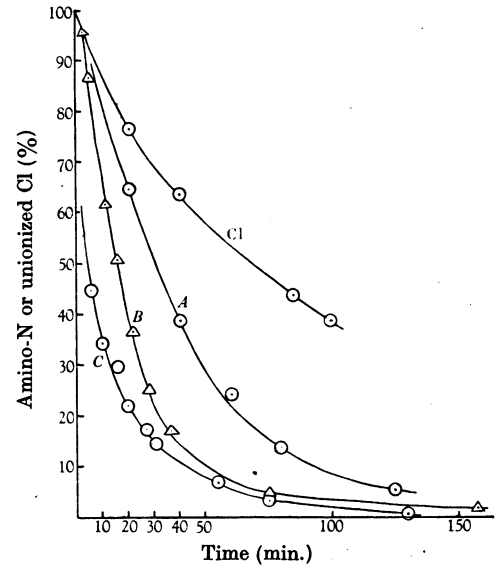


Fig. 1. The reaction with glycine at pH 7·5 and 38° of (*A*) *H*-sulphone, (*B*) *H*-sulphone previously treated with $NaHCO_3$ at pH 7·5 and (*C*) divinyl sulphone. (Cl, ionic chlorine liberated from the *H*-sulphone in $NaHCO_3$ solution at pH 7·5.)

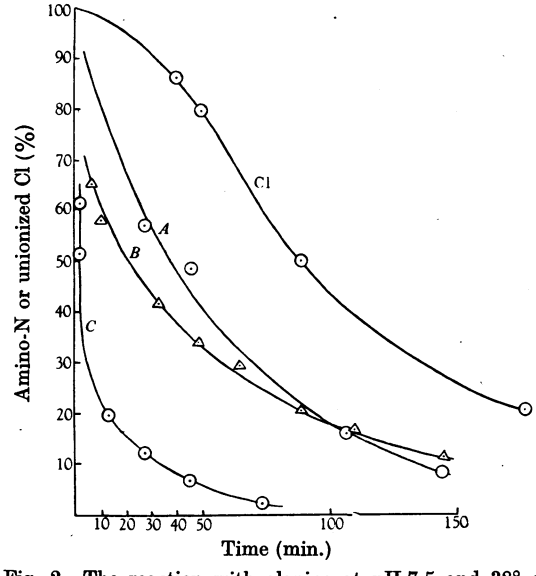


Fig. 2. The reaction with alanine at pH 7·5 and 38° of (*A*) *H*-sulphone, (*B*) *H*-sulphone previously treated with $NaHCO_3$ at pH 7·5, and (*C*) divinyl sulphone. (Cl, ionic chlorine liberated from the *H*-sulphone in $NaHCO_3$ at pH 7·5.)

it was decided to compare the rates of action of the two sulphones on amino-acids by measuring the

decrease in amino-N (formol method). Furthermore, since Ford-Moore (1940) and Ford-Moore & Lidstone (1940) have shown that HO_2 in alkaline solution loses HCl to give β-chloroethyl-vinyl sulphone, and then divinyl sulphone, and have suggested that this removal of the HCl precedes the reaction of HO_2 with amino-groups, we included in our experiments a test with HO_2 previously stirred with bicarbonate buffer at pH 7·5 until most of its chlorine had become ionized. To make the tests strictly comparable, the divinyl sulphone also was stirred at pH 7·5 for the same length of time. 1·5 mol. sulphone/mol. of amino-acid were used and the experimental details were as follows:

	0·75 M-$NaHCO_3$-H_2CO_3 (pH 7·5) (ml.)	Sulphone (in 60 ml. of ether)
A	40	2·86 g. of HO_2
B	40	2·86 g. of HO_2
C	40	1·77 g. of divinyl sulphone

Mixtures B and C were prepared and were stirred vigorously at 38° for 110 min., during which period 64 % of the Cl in mixture B became ionized. Mixture A was then prepared, and 10 ml. of M-glycine in 0·75 M-$NaHCO_3$ solution was added to each mixture (A, B and C). These mixtures were stirred vigorously at 38°, and samples (2 ml.) were withdrawn from each at intervals. The samples were cooled in ice, made acid with conc. HCl (4 drops), freed from CO_2 as quickly as possible, and amino-N determinations (by the technique described in the experimental part of this paper) carried out immediately.

The results of this experiment (Fig. 1) showed that the diminution in amino-N is much more rapid with divinyl sulphone than it is with HO_2, and that preliminary stirring of the HO_2 in the bicarbonate buffer produced a mixture which acted on glycine almost as rapidly as did divinyl sulphone.

A similar experiment (Fig. 2) was carried out with alanine, the technique being the same as that described above, with minor differences (50 ml. of the bicarbonate buffer was used and the preliminary stirring period for two of the mixtures was 3¼ hr., during which time about 80 % of the Cl of the HO_2 became ionized; 0·7 M-alanine in bicarbonate buffer was used).

EXPERIMENTAL

Materials

Mustard gas (H). The *H* used in the work described in this and the following papers was obtained from the War Office and later the Ministry of Supply. It was redistilled twice.

ββ′-Dichlorodiethyl sulphone (HO_2). Prepared from *H* by the method of Helfrich & Reid (1920).

Divinyl sulphone. Prepared from HO_2 by the method of Dr E. Walker as described by Alexander & McCombie (1931).

Divinyl sulphide. Freshly prepared before use, by the method of Bales & Nickelson (1922, 1923).

Methods

Amino-N determinations. The reaction of the sample was adjusted to pH 7 (neutral red) and the solution was titrated with 0·05 N-NaOH to pH 9 (phenolphthalein); excess of neutralized 20 % formaldehyde solution was added and the mixture was again titrated to pH 9. The amino-N was calculated from the total amount of NaOH required for the two titrations; occasionally the determination was made as a one-stage operation by omitting the first titration to pH 9. When $NaHCO_3$ or Na_2CO_3 was present in the original solution, the sample was first made distinctly acid with HCl (e.g. 4 drops of conc. HCl to a 2 ml. sample in many of the experiments) and evacuated several times to remove CO_2, and the reaction of the solution brought to pH 7.

Micro-analyses. These were carried out by Dr Weiler, or in the Cambridge University Chemical Laboratory (by courtesy of Dr H. McCombie).

Action of mustard gas on amino-acids and their esters

On glycine ester. Following the method of Cashmore & McCombie (1923), 26·6 g. of glycine ester hydrochloride (0·19 mol.), 8·9 g. of anhydrous Na_2CO_3 (0·084 mol.), and 71 ml. of 97 % ethanol were mixed, warmed on a water-bath for a few minutes, and 14·2 g. of anhydrous Na acetate (0·17 mol.) with 14·2 g. of *H* (0·089 mol.) added. The mixture was heated for 4½ hr. on a water-bath, filtered, and the precipitated NaCl, etc., washed with 97 % ethanol. The combined filtrate and washings were concentrated *in vacuo* to about 40 ml. and poured into 225 ml. dilute HCl (containing 150 ml. of 2N-acid). The solution was extracted with ether to remove unchanged *H*, brought to about pH 8 with solid $Na_2CO_3.10H_2O$, and again extracted with ether. The ethereal solution was dried with Na_2SO_4, the ether removed on a water-bath, and the residue distilled *in vacuo*. Three fractions were obtained: (i) b.p. 145–158°/15 mm. (3·06 g.); (ii) b.p. 190–193°/15 mm. (this fraction, weighing 2·60 g., set to a jelly-like mass on cooling); (iii) a residue (1·59 g.) not distilling at 5 mm. with a bath temperature of 200°. Cashmore & McCombie (1923) reported the b.p. of their ester as 159–160°/15 mm.

Fractions (ii) and (iii) contained sulphur but very little nitrogen, and gave neither picrates nor platinichlorides. It was presumed that these were mainly divinyl sulphide polymers, and they were rejected.

Addition of 10 % platinum chloride solution to a solution of fraction (i) in ethanol gave an amorphous platinichloride, insoluble in water and alcohol, containing 34·35 % Pt (Cashmore & McCombie recorded 34·9 %). Clarke (1912) showed that many amorphous alkyl thiazan platinichlorides have the structure $B.HPtCl_5$. A *platinichloride* of this type, derived from *ethyl* 1:4 *thiazan-4-acetate*, would have a Pt content of 34·7 %.

The *picrate* of the ester, prepared from fraction (i) in ethanol solution and recrystallized from absolute ethanol, had m.p. 170°. (Found: C, 41·5; H, 4·4; N, 13·3; S, 7·8. The picrate of the thiazan, $C_8H_{15}NO_2.C_6H_3N_3O_7$, requires C, 40·2; H, 4·3; N, 13·4; S, 7·7 %.)

The ester was hydrolyzed by boiling with 7 ml. N-NaOH/g. of ester for 3 hr. The copper salt of the acid was then precipitated by addition of 7 ml. of 0·5 M-$CuSO_4$/g. of ester, and

decomposed by suspending in water at 80° and saturating with H_2S. After filtration and aeration, to remove excess H_2S, the solution was evaporated *in vacuo* over H_2SO_4 (yield 1·87 g.), and the product crystallized from absolute ethanol. The acid appeared to be dimorphous, forming either short thick prisms with wedge-shaped ends, or fine flat needles; m.p. of both forms (and mixed m.p.) 175–176·5°, after shrinking and softening at 133°. The substance, particularly in the crude state, was hygroscopic, crystallization sometimes being difficult. (Cashmore & McCombie (1923) record m.p. 132° for their product and a crystalline form of flat plates.) Analytical figures on the product, after drying over $CaCl_2$ or P_2O_5, were not very satisfactory, but appeared to conform most nearly to the structure of 1:4-*thiazan*-4-*acetic acid*, with one molecule of water of crystallization. The acid could not be titrated satisfactorily in aqueous solution, in contrast to the corresponding HO_2 derivative (*vide infra*), and furthermore there was no free amino-N titratable by the formol method. In absolute ethanol solution, however, titration with N-NaOH gave a sharp end-point with phenolphthalein, corresponding to a mol. wt. of 181 (method of Willstätter & Waldschmidt-Leitz, 1921). The thiazan monohydrate requires a mol. wt. of 179. Prolonged drying at 56° *in vacuo* over P_2O_5 caused a loss of weight of 10·1 % (theoretical for the monohydrate) after 18 hr. (Found, on the dried material: C, 44·7; H, 7·1; N, 8·7; S, 19·8. $C_6H_{11}O_2NS$ requires C, 44·7; H, 6·9; N, 8·7; S, 19·9 %.) M.p. 176–176·5°, without preliminary softening. Further drying *in vacuo* over P_2O_5 at 78° caused an additional loss of weight of 3·5 %, with slight decomposition.

The *picrate* of the free acid, prepared in ethanol solution and recrystallized from ethanol, had m.p. 175–176°. The analytical figures (C, 39·2; H, 4·6; N, 13·3 %) correspond to a formula of one molecule of the thiazan with one molecule of picric acid and one molecule of ethanol, but no loss of weight was observed on drying *in vacuo* over P_2O_5, either at 56° or 78°. The *sulphilimine*, prepared by the addition of 200 mg. of chloramine-T in 2·3 ml. of water to 100 mg. of the thiazan in 0·3 ml. of water, after two recrystallizations from 1 ml. water, had m.p. 123·5°. (Found: C, 48·7; H, 5·4; N, 8·4; S, 18·8. $C_{13}H_{18}N_2S_2O_4$ requires C, 47·3; H, 5·5; N, 8·5; S, 19·4 %.)

From these results, two structures could be assigned to the compound: (i) the thiazan structure with one molecule of water of crystallization, or (ii) β-hydroxy-β′-glycinodiethyl sulphide. The absence of —NH— or —OH groups, as indicated by the fact that the compound does not react either with nitrous acid to form a nitrosamine or with 3:5-dinitrobenzoyl chloride, appears to exclude the latter. The structure 1:4-thiazan-4-acetic acid is therefore attributed to the compound, which is usually referred to for convenience as '*H*-glycine'.

On alanine ester. An attempt was made to prepare from alanine ester hydrochloride a derivative analogous to the thiazan derivative of glycine described above. The procedure was similar to that for the preparation of '*H*-glycine', but although there was undoubtedly some reaction, involving a 60 % reduction in the amount of amino-N in the mixture (formol titration), no crystalline material could be separated from the intractable oil obtained.

On cysteine. 30 mg. of cysteine hydrochloride was dissolved in 0·5 ml. of N-NaOH in a stoppered tube and 1·6 ml. of 0·5 M-$NaHCO_3$ and 1 drop of *H* added. The mixture was shaken gently at intervals and samples were tested for free SH by the nitroprusside test; these tests showed significant loss of SH in 5 min. with little free SH left 15 min. and none 30 min. after the start of the reaction. A control test, with no *H*, showed no loss of SH in 30 min. and very slight loss after 55 min.

On arginine. A mixture of 18 ml. of a 0·57 M-solution of arginine in 0·375 M-$NaHCO_3$ buffer (pH 7·5) was stirred for 16 hr. at 37° with 0·8 g. of *H* added gradually at fairly regular intervals, with a control experiment without *H*. Amino-N determinations showed 12·5 % loss of amino-N in 2½ hr., 40 % in 9½ hr. and 57 % in 16½ hr. Although the formol method does not give a theoretical titration figure with arginine, these results indicate a definite decrease in the free amino-N of the arginine and are in conformity with the observation of Hellerman (1942) that *H* reacts with the α-amino-groups of arginine. No comparable reaction has been observed in our similar experiments with alanine.

Other attempts to condense H (and divinyl sulphide) with amino-acids

Several unsuccessful efforts have been made to condense *H* with various amino-acids, sometimes under mild conditions (pH 7·5–8·5 and at 30–40°) and sometimes at higher temperatures and in a very alkaline medium. No copper derivative other than that of the unchanged amino-acid could be isolated, for example, from preparations obtained (*a*) when glycine or glutamic acid was boiled for 21 hr. in aqueous ethanol with *H*, anhydrous Na_2CO_3 (or anhydrous Na acetate or $CaCO_3$), or (*b*) when alanine was heated with *H*, Na acetate and 97 % ethanol for 4½ hr. with subsequent treatment as in the preparation of '*H*-glycine'. Furthermore, a solution of alanine in bicarbonate buffer at pH 7·5–8·5 showed no reduction in titratable amino-groups (formol method) when stirred at 30° for 12½ hr. with *H*, and with glycine no copper derivative could be separated from the mixture even after 29 hr.

A few experiments have been carried out with divinyl sulphide and glycine, to run parallel with the investigation of the action of this reagent on proteins (Boursnell *et al.* 1946). A mixture of divinyl sulphide and glycine in bicarbonate buffer at pH 7·5 stirred at 30° for 9 hr. showed no reduction of free amino-N, nor could a copper salt, other than copper glycine, be isolated from the product obtained when divinyl sulphide was heated for 4 hr. with glycine ester hydrochloride and Na_2CO_3 in 97 % ethanol.

Action of H-sulphone (HO_2) on amino-acids

'*HO_2-glycine*' (1:4-*sulphonazan*-4-*acetic acid*). 0·4 g. of glycine dissolved in 20 ml. of 0·5 M-$NaHCO_3$, and 0·95 g. of HO_2 dissolved in 25 ml. of ether, were stirred together at 30° for 11 hr. with occasional additions of NaOH to maintain the pH at about 8·5. (Later experiments showed that the reaction was practically complete in 3 hr.) The solution was neutralized with HCl (to phenol red), and the product isolated via the copper salt as described for '*H*-glycine'. The resulting colourless crystals (0·74 g.; 77 % yield), after three recrystallizations from 50 % ethanol, and drying over P_2O_5 *in vacuo*, had m.p. 181°.

Many micro-analyses were carried out on preparations of 'HO_2-glycine' (prepared as above and by the methods of Cashmore & McCombie, 1923, and Lawson & Reid, 1925, and having identical melting-points and mixed melting-points) with varying results, generally corresponding with $C_6H_{11}O_4NS$ plus one-third to one molecule of water. A

sample dried over P_2O_5 at room temperature for a short time only gave C, 34·3; H, 6·1; N, 6·3; S, 15·4%. (Theory for monohydrate: C, 34·1; H, 6·2; N, 6·6; S, 15·2%.) A specimen dried over $CaCl_2$ only, gave C, 34·4; H, 6·1; N, 6·5%; this sample, dried over P_2O_5 *in vacuo* at 56° for 10 hr., then at 78° for a further 75 hr., lost 7·85% of its original weight (theory for $1H_2O$, 8·54%). The rate of loss of weight after this time was extremely small, about 0·01% per day, and the dried crystals on analysis gave C, 37·2; H, 6·3; N, 6·9; S, 16·8%. (Calc. for $C_6H_{11}O_4NS$, C, 37·3; H, 5·7; N, 7·3; S, 16·6%.)

These analyses, like those of the corresponding *H* derivative, indicate firm retention of a molecule of water, though this has not previously been reported. The possibility of the β-hydroxy-β′-glycino-diethyl sulphone structure seems to be precluded by the relative ease with which a portion of this molecule of water is lost, and by the absence of any titratable primary or secondary amino-groups (formol titration). The ring structure also seems more probable, by analogy with other HO_2-amino-acid compounds whose analyses all agree well with the anhydrous thiazan structure.

'*HO_2-alanine*' (1:4-*sulphonazan*-4-α-*propionic acid*). (*a*) *In boiling solution.* Following the method of Lawson & Reid (1925), equimolecular quantities (0·0167 mol.) of HO_2, alanine, and anhydrous Na_2CO_3 were boiled with 25 ml. of water for 5 hr. The solution was neutralized with HCl and the product isolated via the copper salt. After one crystallization from 50% ethanol the resulting short colourless prisms had m.p. 186° (yield 45%). (Found, after drying the thrice-recrystallized preparation over P_2O_5 *in vacuo*: C, 41·0; H, 6·3; N, 6·9; S, 15·7. $C_7H_{13}O_4NS$ requires: C, 40·6; H, 6·3; N, 6·8; S, 15·5%. Mol. wt. by titration in aqueous solution (phenolphthalein), 211; theory, 207. No free NH_2 or —NH— group (formol titration).) (*b*) *At* 30°. 0·0022 mol. of alanine, dissolved in 15 ml. of 0·5 M-$NaHCO_3$, was stirred with 0·0052 mol. of HO_2 dissolved in ether, with occasional addition of NaOH to maintain the pH at about 8·5. After 6 hr. the product was isolated via the copper salt and recrystallized from 50% ethanol. Yield about 80 mg. (17% of theory); m.p. and mixed m.p. with a sample from preparation (*a*), 186°.

'*HO_2-phenylalanine*' (1:4-*sulphonazan*-4-α-*benzylacetic acid*). 0·05 g. of phenylalanine was dissolved in 5 ml. of 0·5 M-$NaHCO_3$, and added to 0·06 g. of HO_2 dissolved in ether. After several days at room temperature, with occasional shaking, the solution was neutralized and the product isolated via the copper salt. The resulting colourless crystals (38·5 mg.; 45% yield), after two crystallizations from water, had m.p. 173°; a mixed melting-point with a sample prepared according to the Lawson & Reid (1925) method showed no change.

'*HO_2-tyrosine*' [1:4-*sulphonazan*-4-α-(p-*hydroxybenzyl*) *acetic acid*]. 1·35 g. of tyrosine were suspended in 30 ml. of 0·5 M-$NaHCO_3$, and dissolved by addition of 15 ml. of N-NaOH and 45 ml. of water. The solution was stirred at 30° for 12 hr. with an ethereal solution of 1·43 g. of HO_2. Occasional addition of 30% NaOH was required to keep the tyrosine in solution. A trace of unchanged tyrosine was removed by saturating the solution with CO_2, and the product was precipitated by acidification to methyl red with HCl, redissolved in N-NaOH and reprecipitated with N-HCl. The dried material (1·51 g.; 68% yield) was recrystallized from 50% ethanol, producing clusters of needles; m.p. 220·5–221·5°, with charring. (Found, after drying the thrice-recrystallized specimen over P_2O_5 *in vacuo* at 50°: C, 52·2; H, 5·8; N, 4·9; S, 11·0. $C_{13}H_{17}O_5NS$ requires: C, 52·1; H, 5·7; N, 4·7; S, 10·7%. Mol. wt., by titration in aqueous solution (phenolphthalein), 301; theory, 299. No free NH_2 or —NH-group (formol titration).)

Action of HO_2 on cysteine. In an experiment carried out under conditions similar to those of the experiment with *H*, 120 mg. of HO_2 were allowed to act on 30 mg. of cysteine hydrochloride in slightly alkaline solution. A decrease in SH was noted almost immediately, and samples taken after 5 min. showed no free SH. It is of interest to note that Ford-Moore (1940) obtained ββ′-*bis*-(2-carboxy-2-aminoethylthio)-diethyl sulphone by the action of divinyl sulphone on cysteine hydrochloride.

Action of divinyl sulphone on glycine

0·0049 mol. of glycine in 15 ml. of 0·5 M-$NaHCO_3$ and 0·0051 mol. of divinyl sulphone dissolved in 15 ml. of ether were stirred together at 30° for 7 hr. The product was isolated via the Cu salt (wt. 0·74 g.; 75% yield), and the recrystallized compound had m.p. 181°; mixed m.p. with 'HO_2-glycine', 181°.

DISCUSSION

The action of *H* and divinyl sulphide on amino-acids contrasts strongly with that of *H*-sulphone (HO_2) and divinyl sulphone. Whereas the two sulphones react readily under physiological conditions with the α-amino-groups of all the amino-acids studied, yielding in every case a derivative with a 1:4-sulphonazan structure, no similar reaction has been observed with either *H* or divinyl sulphide, except possibly in the case of arginine where treatmen twith *H* results in a decrease in amino-N (formol titration). Thus, by analogy, it seems unlikely that the action of *H* on proteins can be attributed to a general reaction with free amino-groups. *H* does react, however, and very rapidly, with the SH group of cysteine.

Under more drastic conditions, i.e. in boiling ethanol and in the presence of Na acetate, *H* reacts with the amino-group of glycine ester to yield a derivative having a 1:4-thiazan structure, but it seems unlikely that a similar reaction occurs with amino-acids or proteins under physiological conditions.

The rapid action of HO_2 on the α-amino-groups of amino-acids at pH 7·5–8·5 and at 30–40° supports the view that a reaction of this type accounts for the changes effected in proteins by this sulphone, but there is also the probability that HO_2 combines with free SH when this group is present in the protein. The free SH of cysteine is more rapidly destroyed by HO_2 than it is by *H*.

Divinyl sulphone reacts with glycine (and alanine) much more rapidly than does HO_2, but the two sulphones give the same product. A comparison of the rate of action of these two sulphones on glycine or alanine with the rate of action of HO_2 which has previously been stirred in bicarbonate solution at

38°, offers strong support for the view that HO_2 loses one or two molecules of HCl to give an unsaturated compound before acting on amino-groups. The curves (Figs. 1, 2) showing the decrease in the amino-N of the amino-acid when treated with HO_2 certainly showed a marked resemblance to the rate of liberation of ionized Cl from HO_2 stirred in bicarbonate at pH 7·5, and the 'hydrolyzed' product obtained by the latter process showed a capacity to react with the amino-group of glycine or alanine nearly equal to that of divinyl sulphone.

SUMMARY

1. The actions of mustard gas (*H*), $\beta\beta'$-dichlorodiethyl sulphone (HO_2), divinyl sulphide and divinyl sulphone on some amino-acids at pH 7·5–8·5 and at 30–40° have been studied.

2. HO_2 rapidly reacts under these conditions with the amino-groups of amino-acids to give derivatives containing the 1:4-sulphonazan ring. The hitherto undescribed 'HO_2-alanine' (1:4-sulphonazan-4-α-propionic acid) and 'HO_2-tyrosine' [1:4-sulphonazan-4-α-(*p*-hydroxybenzyl)-acetic acid] have been prepared in this way. HO_2 also reacts rapidly with the SH groups of cysteine.

3. *H* and divinyl sulphide do not appear to have any similar action on amino-groups under physiological conditions. *H* quickly abolishes the free SH of cysteine, and it more slowly reduces the amino-N (formol determination) of arginine.

4. Under more drastic conditions, in boiling ethanol in the presence of Na acetate, *H* reacts with the amino-groups of glycine ester to give a new reaction product, ethyl 1:4-thiazan-4-acetate; on hydrolysis this ester yields 1:4-thiazan-4-acetic acid. The preparation and properties of some derivatives of the ester and of the acid are described.

5. Divinyl sulphone reacts under physiological conditions with glycine (or alanine) even more rapidly than does HO_2, the two sulphones giving the same product. Determination of the rate of diminution of free amino-N in the reacting mixtures supports the view that HO_2 is converted into divinyl sulphone before it reacts with amino-acids and proteins.

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