ANTIBIOTIC PRINCIPLE OF *ALLIUM SATIVUM*

The Structure of Allicin ; Preparation and Properties of Diphenyl Disulphide Oxide

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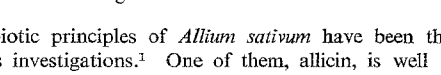
SUMMARY

The constitution of allicin, the antibiotic principle of *Allium sativum* is discussed and a diallyl disulphide oxide structure is suggested in place of the diallyl thiolsulphinate formulation by Cavallito and co-workers. The aromatic analogue diphenyl disulphide oxide has been prepared and its properties are recorded. The antibacterial activity of a few sulphur-containing substances is given.

The antibiotic principles of *Allium sativum* have been the subject of many previous investigations.[1] One of them, allicin, is well characterised and has been shown by Cavallito and co-workers to be highly inhibitory to the growth of gram positive and gram negative bacteria.[1] It is also active against *Mycobacterium Tuberculosis* and certain fungi.[2] Cavallito *et al.*, have suggested a 'diallyl thiolsulphinate' structure[3] (I) for it.



(I) (II)

($R_1 = R_2 =$ allyl)

Despite the wide range of its anti-microbial activity *in vitro*, allicin and a few synthetically prepared compounds of this group do not appear to have proved so far successful therapeutically, presumably owing to the high toxicity of allicin. Of the compounds belonging to this group, no derivative with aromatic substituents has been previously investigated and it was considered desirable to study representatives of this group ($R_1 = R_2 =$ phenyl, etc.) and also of unsymmetrically substituted 'thiolsulphinates'. For this purpose, a preparative method which may incidentally provide an unequivocal confirmatory evidence for the 'thiolsulphinate' structure of allicin, is necessary as the oxidation of the unsymmetrically substituted disulphides with per-acids obviously would lead to mixtures of 'thiolsulphinates' difficult to separate.

Three methods schematically represented below have been tried but invariably very little of the expected final product is formed.

(*a*) (i) $R \cdot MgCl + SOCl_2 \rightarrow R \cdot SO \cdot Cl + MgCl_2$

(ii) $R \cdot SO \cdot Cl + R'SM \rightarrow R \cdot SO \cdot S \cdot R' + MCl$

(*b*) (i) $RSH + SOCl_2 \xrightarrow[\text{(Pyridine)}]{} R \cdot S \cdot SO \cdot Cl + Py \cdot HCl$

(ii) $R.S.SO \cdot Cl + R \cdot MgBr \rightarrow R \cdot S \cdot SO \cdot R + MgBrCl$

(*c*) (i) $R \cdot S \cdot Ag + SOCl_2 \rightarrow R \cdot S \cdot SO \cdot Cl + AgCl$

(ii) $R \cdot S \cdot SO \cdot Cl + R \cdot MgBr \rightarrow R \cdot S \cdot SO \cdot R + MgBrCl$

R = R′ = allyl or phenyl ·

M = a metal, silver or sodium.

By the method (*a*), which was also used by Cavallito and Bailey,[4] a product, no doubt possessing a low antibacterial activity was obtained but it could not have contained even traces of allicin as it did not liberate iodine with hydriodic acid. The above reactions were also carried out using the corresponding phenyl derivatives instead of the allyl (R = phenyl). In this case the end-products were identified as diphenyl disulphide and diphenyl sulphide. Traces of high melting substances probably polymeric in character, were also formed. The course of these reactions appears to be in accordance with the work of Oddo and of Strecker[5] and may be represented by the following equations:—

(*d*) $RSH + SOCl_2 \rightarrow R \cdot S \cdot SO \cdot Cl + HCl$

(*e*) $RSH + R \cdot S \cdot SO \cdot Cl \rightarrow R \cdot S \cdot SO \cdot SR + HCl$

(*f*) $R \cdot S \cdot SO \cdot Cl + RMgBr \rightarrow R \cdot S \cdot SO \cdot MgBr + RCl$

(*g*) $R \cdot S \cdot SO \cdot MgBr + RMgBr \rightarrow R—S—S—R + (MgBr)_2O$

(*h*) $R \cdot S \cdot SO \cdot SR + RMgBr \rightarrow R \cdot S \cdot SO \cdot MgBr + R—S—R$

It has not been found possible, thus, to provide a direct synthetic evidence for the ' thiolsulphinate ' structure of allicin. The diphenyl analogue (m.p. 69°) was, however, prepared by oxidation of diphenyl disulphide with perbenzoic acid and it has been found to be similar to allicin in its properties. It is a highly crystalline substance and in this respect is unlike any previously described. In the course of study of this substance the evidence presented by Cavallito, *et al.*, for the structure of allicin was fully examined and is discussed below.

Structure of Allicin.—Cavallito, Buck and Suter[3] first considered five theoretically possible structures, three of which were eliminated on the

strength of its reaction with cysteine. Of the remaining two, structure (I) was favoured as it was thought (i) its solubility in water; (ii) agreement of its observed molecular refraction with the calculated figure, and (iii) its formation from the disulphide by oxidation with perbenzoic acid, could be satisfactorily explained.

However, structure (I) shows the calculation of molecular refraction by Cavallito, *et al.*, is not quite correct in the sense that the atomic refraction of oxygen has been assumed to be 1·982 which is the figure for atomic refraction in the *liquid state*. Further they have not taken into consideration the functional significance of oxygen on the constitutive influence of the group —S—S—, on molecular refraction, undoubtedly due to lack of necessary
↓
O
data in literature. These facts, therefore, leave their calculated figure for molecular refraction uncertain and this evidence loses much of its significance in relation to structure (I).

The formation of allicin by oxidation of diallyl disulphide with perbenzoic acid may make structure (II) improbable but structure (I) is thereby not definitely established. In analogy with the mechanism of epoxidation[6] of unsaturated carbon bonds, a structure like R—S=S—R can also be
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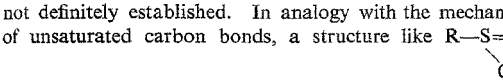
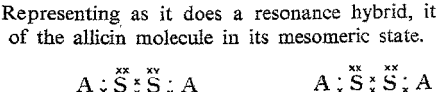
pictured in this case, as shown below, for there are available unshared electrons on the two sulphur atoms. In a structure like this, the octet rule of Lewis need not necessarily hold good in case of elements like sulphur.[7]

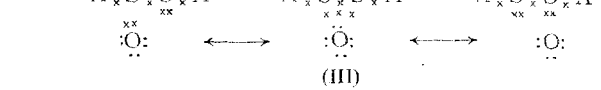


It could be seen that as the two sulphur atoms are symmetrically disposed, all the conditions necessary for resonance[8] between structures (I *a*) and (I *b*) are fulfilled and hence the structure of allicin in its mesomeric state should be in-between these two and may be better represented by structure (III). Representing as it does a resonance hybrid, it should give a truer picture of the allicin molecule in its mesomeric state.



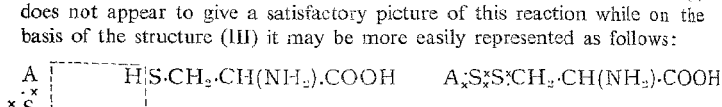
(I *a*) (I *b*)

Or



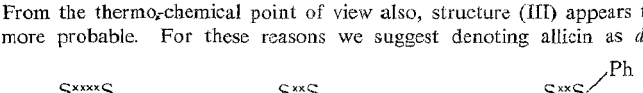
(III)

On the basis of this concept, not only all the reactions of allicin could be satisfactorily explained but also it provides a better explanation of the almost quantitative formation of compound Allyl—S—S—CH_2—CH—COOH
|
NH_2
(IV) from allicin and cysteine which, *prima facie* involves a reaction of two sulphur atoms similarly. This reaction takes place under conditions of comparative stability of allicin (at pH 6·0 and in the cold) and it may be rather difficult to postulate a disproportionation of the molecule prior to combination with cysteine. An unsymmetrical representation such as (I) does not appear to give a satisfactory picture of this reaction while on the basis of the structure (III) it may be more easily represented as follows:



(A = allyl)

The determination of the dipole moment of the diphenyl analogue of allicin has not given us conclusive evidence in favour of either of the two, but the observed high figure ($\mu = 3{\cdot}8$ D; in benzene) appears to satisfy both these structures qualitatively. It has not been possible to calculate accurately the dipole moments on the basis of these structures, as the relevant data on sulphur-oxygen bonds could not be obtained by us in literature. From the thermo-chemical point of view also, structure (III) appears to be more probable. For these reasons we suggest denoting allicin as *diallyl*



(III *a*) (I)

disulphide oxide rather than ' diallyl thiolsulphinate '.

Owing to the close similarity between the 'thiolsulphinates' and the thiolsulphonates, *p*-acetamino-*p'*-nitro-diphenyl-thiolsulphonate, as also some other already known sulphur containing compounds, were prepared and their antibacterial activity ascertained *in vitro* with the hope that they could provide models for further work. The compound *p*-acetamino-*p'*-nitrodiphenyl-thiolsulphonate has not been previously described in literature. The details are given in the experimental part.

Experimental

Diphenyl disulphide oxide was prepared by oxidising diphenyl disulphide,[9] with perbenzoic acid[10] in a manner somewhat similar to that described by Cavallito and Bailey[2] for the preparation of 'diallyl thiolsulphinate'.

To a solution of diphenyl disulphide (0·1 mol.) in dry chloroform (50 c.c.) maintained at 0° was slowly added during half an hour, a chloroform solution containing perbenzoic acid (0·1 mol.) under mechanical stirring. After the addition was over, the reaction mixture was further stirred for 50 minutes at room temperature (25° C.) after which it was shaken with an ice-cold aqueous solution of sodium bicarbonate (0·1 mol.; 5%) followed by 50 c.c. of 2% solution. The chloroform solution was washed finally with ice-cold water (50 c.c.), quickly dried over anhydrous sodium sulphate and evaporated to about 10 c.c. under suction. After addition of petroleum-ether (30°–50°; ca. 50 c.c.), the mixture was cooled in ice-salt bath, scratched with a glass rod and kept in an ice-chest for the next twelve hours. Clusters of pale yellow slender needles that separated were collected and recrystallised from petrol. The substance (yield, 23% of the theoretical), melted at 69°, which did not change on recrystallisation from the same solvent (Found: C, 60·83; H, 4·12; S, 27·48%. $C_{12}H_{10}S_2O$ requires C, 61·50; H, 4·3; S, 27·36%).

It is easily soluble in alcohol, ether, chloroform and sparingly in petrol. It decomposes with darkening at about 101° when heated in a melting point capillary. The substance resembled allicin in its reaction with hydriodic acid as shown below.

To a solution of the above substance (0·0234 gm.) in chloroform (2 c.c.) contained in a 25 c.c. glass-stoppered bottle, a mixture of 2 c.c. aqueous potassium iodide (5%) and 2 c.c. of sulphuric acid (20%) was added and the bottle shaken vigorously for three minutes and cooled in ice. The liberated iodine was titrated in the usual way with sodium thiosulphate (N/20) using a dilute aqueous solution (2 drops) of starch as indicator. Only 0·42 c.c. of the thiosulphate solution was consumed as against the theoretical requirement of 4 c.c. Thus the amount of iodine liberated was only 10·2% of the

theoretically (based on the amount of substance taken) expected value. In a blank run simultaneously with chloroform (2 c.c.), aqueous potassium iodide (2 c.c.; 5%) solution, and sulphuric acid solution (20%; 2 c.c.) practically no iodine was liberated on shaking for the same length of time.

Dipole moment was determined in benzene solution (conc. = 2·869%) at temperatures 30° and 40° and as calculated according to Debye's equation the mean value is found to be $\mu = 3{\cdot}8$ D.

p-*Acetamino*-p'-*nitro-diphenyl thiolsulphonate* was prepared according to the general method of Miller and Smiles,[11] used by them for the preparation of other thiolsulphonates.

Thoroughly dried *p*-acetaminobenzene sulphonyliodide[12] (6·8 gm.) was dissolved in dry benzene (100 c.c.) and kept boiling in a three necked flask fitted with a mechanical stirrer. Silver *p*-nitrothiophenol (5·46 gm.) was added gradually with stirring over 15 minutes. The source of heat was then removed but the mixture kept stirred vigorously for a further period of quarter of an hour. The benzene layer was separated from the precipitated silver iodide and concentrated to half its volume. After keeping at room temperature (28°) for 24 hours, the crystalline material that separated was removed and recrystallised from hot benzene. It melted at 174°. (Found: C, 36·62; H, 26·40; N, 5·98; S, 13·79%. $C_{14}H_{12}O_5N_2S_2$ requires C, 36·45; H, 26·225; N, 6·07; S, 13·90%). The substance is sparingly soluble in alcohol and cold benzene but dissolves well in acetone.

Antibacterial Tests.—The antibacterial tests were carried out according to the procedure described previously.[13]

TABLE

Antibacterial Activity in vitro

Organisms	Maximum dilution of the substance for complete inhibition of growth				
	Diphenyl disulphide oxide	*p*-Acetamino, *p*-nitro diphenyl thiolsulphonate	Na. *p*-acetaminophenyl thiolsulphonate[14]	(*p*-Carboxyphenyl) ethylxanthate[15]	*o*-Tolyl ethylxanthate[15]
	(in thousands)				
Micrococcus pyogenes var. *aureus*	40	<5	20	<5	20
Escherichia coli var. *Communis*	20	<5	<5	<5	<5
Aerobacter aerogenes ..	20	<5	<5	<5	<5
Salmonella typhosa (Rawlings)	10	<5	<5	<5	<5
Salmonella typhi-murrum ..	10	<5	20	<5	<5
Salmonella schottmulleri ..	10	<5	<5	<5	<5
Salmonella enteritidis ..	10	<5	<5	<5	<5

14 and 15 are references as to their preparation.

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