Calculated for $C_9H_9O_6N_3$: C, 42·36; H, 3·56, found: C, 42·44; H, 3·46. The sample was found to be identical with a 2,4-dinitrophenyl derivative prepared from commercial L-alanine. A third fraction was obtained after extrusion of the column. The upper part of the column (yellow) was extracted with methanol. The methanol solution was concentrated *in vacuo* to a small volume. Yellow crystals of a potassium salt precipitated. After acidification in aqueous solution, 2,4-dinitrophenyl-D-serine (m.p. 177–178°) was obtained (yield 0·58 moles);

$$[\alpha]_D^{26} = -4{\cdot}3° \pm 0{\cdot}6° \ (c = 1{\cdot}5\% \text{ in absolute ethanol}^{10}).$$

Calculated for $C_9H_9O_7N_3$: C, 39·87; H, 3·35, equivalent weight 283, found: C, 40·02; H, 3·40, equivalent weight by potentiometric titration, 283, 286. The sample was compared with the 2,4-dinitrophenyl derivative prepared from commercial L-serine. The melting point was the same, the rotation opposite[10]. The racemate prepared from equal amounts of the 2,4-dinitrophenyl derivative of L-serine and our D-serine derivative was in every respect identical with the 2,4-dinitrophenyl derivative prepared from commercial racemic serine[11].

Antibiotic X-1008 was isolated from a submerged culture of *Streptomyces sp.* X-1008 by extracting the whole broth with butanol. The extract was concentrated *in vacuo* and the antibiotic precipitated with petroleum ether. It was successively extracted into methylene chloride and methanol, and then crystallized from a mixture of ethanol and acetonitrile. Antibiotic X-1008 forms cube-like crystals, melting with decomposition at 209–216° (corr.);

$$[\alpha]_D^{27} = -282° \ (c = 1\%, \text{ chloroform}).$$

It gives a melting point depression when mixed with antibiotic X-948. Its UV. absorption spectrum is practically identical with that of antibiotic X-948 and shows the presence of the quinoxaline residue. Barium hydroxide hydrolysis gives quinoxaline-2-carboxylic acid. The IR. spectrum is similar, but differs in details from that of antibiotic X-948. The analyses point to the formula $C_{29}H_{38}O_7N_6S$ (calculated: C, 56·66; H, 6·23; N, 13·67; S, 5·22, found: C, 56·40; H, 6·52; N, 13·69; S, 5·08). These results show that antibiotic X-1008 is structurally related to antibiotic X-948. However, there are only 6 nitrogens in its molecule, as against 7 in antibiotic X-948, indicating that the side chain attached to the quinoxaline residue is of a different nature.

Antibiotic X-1008 is, like antibiotic X-948, a highly toxic compound. It is, however, devoid of trypanocidal activity in mice, when given at the tolerated dose[12].

We are indebted to Dr. AL. STEYERMARK and his staff for the microanalyses and to Dr. A. MOTCHANE and his staff for the spectroscopic data.

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Research Laboratories, Hoffmann-La Roche, Inc. Nutley, New Jersey, USA., June 24, 1957.

ethanol. The 2,4-dinitrophenyl derivatives of D- and L-serine showed a similar behavior: $-4{\cdot}3° \pm 0{\cdot}5°$ and $+4{\cdot}4° \pm 0{\cdot}3$, respectively, in 100% ethanol, $-12{\cdot}1° \pm 1{\cdot}5°$ and $+13{\cdot}0° \pm 2°$, respectively, in 95% ethanol, and $-16{\cdot}0° \pm 1°$ for the D-form in 90% ethanol.

[11] R. R. PORTER and F. SANGER, Biochem. J. *42*, 287, (1948).

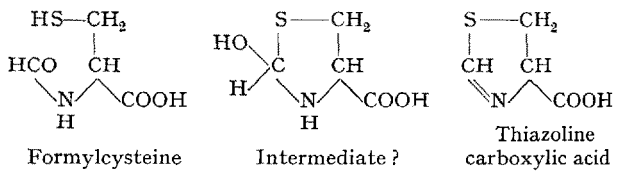
[12] Personal communication by Dr. R. J. SCHNITZER of our Chemotherapy Laboratories.

Zusammenfassung

Es werden zwei neue, chemisch verwandte Streptomyces Antibiotika beschrieben, X-948 ($C_{29}H_{39}O_7N_7S$?) und X-1008 ($C_{29}H_{38}O_7N_6S$?). X-948 ist wahrscheinlich identisch mit Echinomycin. Es hat sehr ähnliche physikalische Eigenschaften und gibt die gleichen Abbauprodukte (Ammoniak, 2-Chinoxalincarbonsäure, N-Methyl-L-valin, L-Alanin und D-Serin).

Thiazoline Carboxylic Acid from Formylcysteine

CALVIN[1] discovered that glutathione (GSH) dissolved in 12 *N* HCl gives the spectrum of a thiazoline derivative with a maximum at 268·5 mμ. By spectrometric evidence a thiazoline derivative was presumed to be produced in the course of the metabolism of thiazolidine carboxylic acid by rat liver preparations[2]. It seemed of interest to assay whether the treatment of N-formylcysteine (FC) with strong acid is followed by the ring closure to a thiazoline derivative in a manner similar to the behaviour of GSH. The expected reaction should follow the steps indicated below:



FC was prepared starting from diformylcystine[3] by the same method used by PIRIE and HELE for the preparation of acetylcysteine[4]. It was dissolved in HCl of increasing normality and the O.D. at 268·5 mμ was registered against the same solvent in function of time.

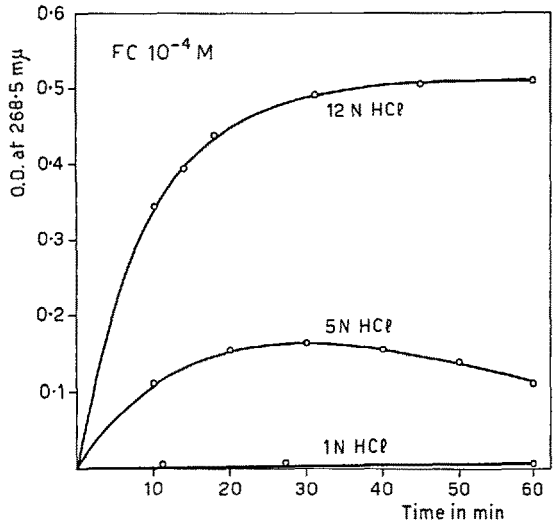


Fig. 1. – Optical density of formylcysteine dissolved in HCl of different strengths at 268·5 mμ, in function of time.

[1] M. CALVIN, *Symposium on Glutathione* (Academic Press, New York 1954).

[2] D. CAVALLINI, C. DE MARCO, B. MONDOVÌ, and F. TRASARTI, Biochim. biophys. Acta *22*, 558 (1956).

[3] V. DU VIGNEAUD, R. DORFMANN, and H. S. LORING, J. biol Chem. *98*, 577 (1932).

[4] N. W. PIRIE and T. S. HELE, Biochem. J. *27*, 1716 (1933).

Figure 1 shows that with the higher concentration of HCl an increase of density is produced, reaching a steady state after 60 min. With the lower concentration of HCl the increase is slower with 5 *N* and nil with 1 *N*. That the production of a thiazoline is dependent on time also for

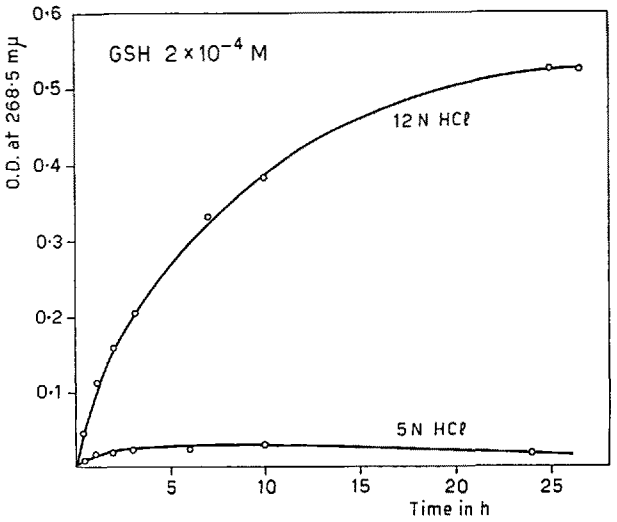


Fig. 2. – Optical density of glutathione dissolved in HCl of different strengths at 268·5 mμ, in function of time.

GSH is shown in Figure 2, which suggests, at the same time, that the behaviour of FC in 12 *N* HCl is of the same type as for GSH, when allowance is made for a difference in speed.

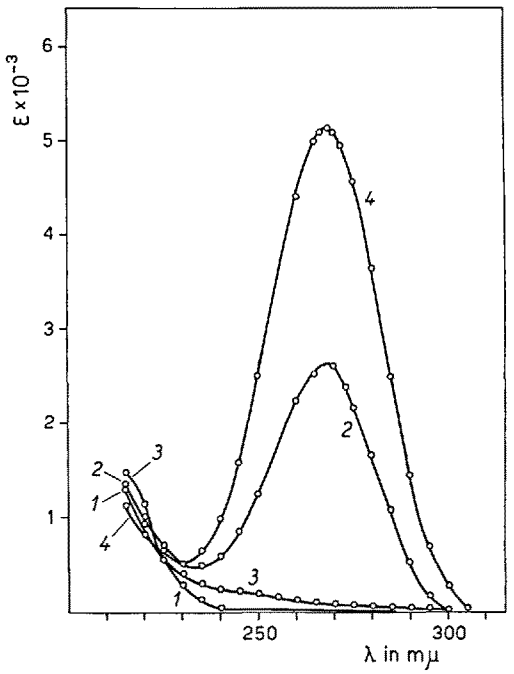


Fig. 3. – Spectrometric curves of glutathione: 1, in water; 2, in HCl 12 *N*; and formylcysteine: 3, in water; 4, in HCl 12 *N*; when the O.D. at 268·5 mμ has reached the maximum (see Fig. 1 and 2).

The complete spectrophotometric curve of FC and GSH in water and 12 *N* HCl, when the higher density at 268·5 mμ is reached, is reported in Figure 3. The curves for FC and GSH have the same shape and equal maximum, suggesting that an equal kind of reaction has taken place for both the compounds. Only a difference in the molar extinction is noted: 5180 for FC and 2625 for GSH.

A sample, of unknown purity, of thiazoline-4-carboxylic acid was prepared as follows. 200 mg of FC were dissolved in 1 ml 12 *N* HCl and left to evaporate over P_2O_5 and KOH pellets in a dessiccator evacuated by a water pump. After a night at room temperature, a mass of crystals was recovered which was rubbed with about 10 ml absolute ethanol and filtered. The insoluble part, which consisted of 20–30 mg of elongated crystals, was dried in vacuum. When dissolved in 12 *N* HCl the compound gave immediately the typical curve shown in Figure 4, with no increase of O.D. at 268·5 mμ with time. The product dissolved in Na_2PO_4 0·1 *M* (pH ca. 8·5) did not give the nitroprusside test for –SH (under the same condition either FC and cysteine gave a strong immediate reaction). The nitroprusside test was positive only when the compound was dissolved in concentrated ammonia and the nitroprusside solution added after a while. These are indications that the thiazoline ring is already present in the compound, that the –SH group is not free, and that it becomes free after hydrolysis in strong ammonia.

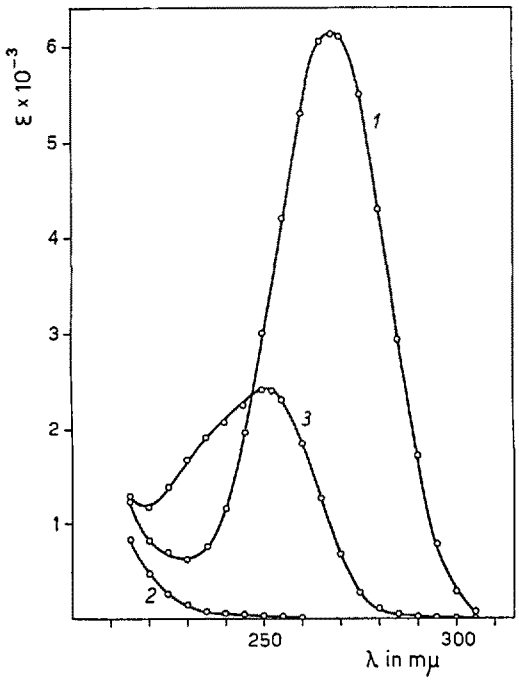


Fig. 4. – Spectrometric curves of thiazoline carboxylic acid, prepared as described in the text: 1, in HCl 12 *N*; 2, in water; 3, in phosphate buffer 0·1 *M* pH 7·4.

The curves in 12 *N* HCl, in water, and in 0·1 *M* phosphate buffer pH 7·4, of the compound prepared as described above, are reported in Figure 4. It is interesting to note that no absorption is shown in water and that at pH 7·4 the maximum is depressed and shifted to the shorter wavelengths. The lack of absorption in water is probably due to hydrolysis of the thiazoline ring in dilute acid medium[5]. Indeed solutions of thiazoline carboxylic acid are acidic and the curve in 5 *N* HCl of Figure 1 suggests that in a not concentrated solution of HCl the production of the thiazoline ring is followed by its rupture.

[5] G. Préaux and R. Lontie, Biochem. J. *66*, 26 p (1957).

The maximum and shape of the curve of thiazoline carboxylic acid in phosphate buffer near the neutrality are very similar to those found for the metabolic product of thiazolidine carboxylic acid by liver preparations[2]. This finding strengthens the hypothesis that a thiazoline derivative is the first product of the biological oxidation of thiazolidine carboxylic acid.

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Riassunto

La formilcisteina in HCl 12 *N* dà un assorbimento nell'ultravioletto indicante la formazione di un anello tiazolinico. La reazione è più rapida per la formilcisteina che per il glutatione e dipende dalla concentrazione dell'acido. Trattando la formicilsteina con HCl 12 *N*, è stato preparato un composto avente le proprietà dell'acido tiazolin carbossilico, che in soluzione di fosfati a pH 7,4 dà una curva spettrofotometrica sovrapponibile a quella ottenuta in un precedente lavoro da un metabolita dell'acido tiazolidin carbossilico. Questo risultato avvalora l'ipotesi che il fegato di ratto è capace di ossidare l'anello tiazolidinico a tiazolinico.

Biochemical Studies on the Degradation Products of Diazinone

During chemical and biochemical studies on some organophosphorus insecticides[1], it was found that diazinone (O,O-diethyl O,2-*iso*propyl-4-methyl-pyrimidyl thionophosphate) is degradated to various derivatives by chemical and other means. It was also observed that plants sprayed with diazinone contained biochemically active residues even after comparatively long periods of time. The results of these experiments are reported in the following:

Methods and material[2]. Plants (*Impatiens balsami*) were sprayed with a 0·02% aqueous diazinone solution, approximately 100 ml per m[2]. Samples (3·5 g of fresh leaves) were taken after various periods of time, and homogenized in water (10 ml). The homogenates were analysed for chemical activity by measuring the cholinesterase-inhibiting effect on an acetylcholine-cholinesterase system with the Warburg technique[3]. The enzyme used was a purified preparation from human blood serum. Before the addition of substrate (acetylcholine chloride), the enzyme was incubated for 50 min with the extracts. Samples taken from unsprayed plants and treated in the same way were used as controls.

Paper chromatograms of various diazinone preparations were carried out with filter paper (Munktell OB) impregnated with silicone or vaseline from a 5% hexane or benzene solution, and air dried. The solvent used was the upper phase from a mixture containing water-ethanol-chloroform (6:10:10)[4]. After air drying, the paper was developed by spraying first with a N-bromo-succinimide solution (0·09 g in 50 ml chloroform and afterwards diluted 1:5 with the solvent) and then with a fluorescein solution (0·33 g in 100 of 0·1 *N* NaOH and afterwards diluted 3:100 with ethanol)[5]. The colour development could only be favorably performed when the paper was treated with vaseline.

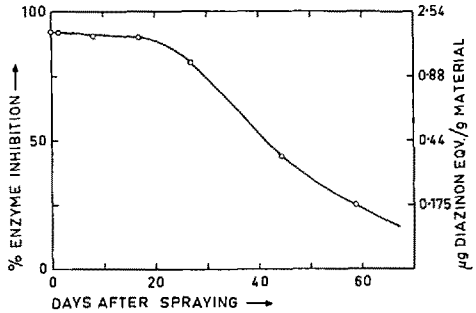

Fig. 1.—Biochemical effect of residues in plants sprayed with diazinone.

Chromatograms not sprayed with the colour reagents were used to study the cholinesterase inhibition by separated constituents. The chromatogram was cut into sections of the same size, usually 20 sections from the starting point to the solvent front, and extracts of each section were made in a bicarbonate buffer solution (the same solution as used in enzyme activity determination[3]). Cholinesterase inhibition by these extracts was assayed using the technique mentioned above. The location of active constituents thus obtained (Fig. 3) was evaluated as per cent enzyme inhibition by each section of the chromatogram, and compared with a chromatogram developed with the colour reagents.

Analysis of residues from diazinone-sprayed plants. The first sample of the plant sprayed with diazinone was taken 1 h after spraying, followed by sampling during one to two months (duplicate series of experiments carried out). The results obtained from one of these experiments are recorded in Figure 1. They indicate that 50% of the active components was present 25 days after spraying. After 9 weeks, the activity of the residues was approximately 20% of the original biochemical activity, corresponding to approximately 0·15 μg of diazinone equivalents per gram plant material. The original amount of diazinone (based on the amount used in spraying) was approximately 10 μg/g material.

It is most probable that diazinone is easily isomerised or converted to analogues (e.g., by oxidation) in a way similar to that demonstrated for parathion[4]. Some of these isomers and analogues might be more active biochemically than diazinone, in the same way as was found for parathion. In order to find support for this view, the following experiments were performed.

Enzyme inhibition by diazinone preparations treated in various ways. The cholinesterase inhibiting activities of pure (99·9%) diazinone and of a one year old preparation containing 95% pure diazinone (chemical analysis) differed greatly, as demonstrated in Figure 2. Even after keeping for four months at room temperature, a preparation behaved much the same as the one year old prepara-

[1] K.-B. AUGUSTINSSON, Acta agr. Scand. *7*, 165 (1957).

[2] We are grateful to Dr. SIV RENVALL of the Swedish Plant Protection Institute, Stockholm, for carrying out the spraying of the plants and taking samples for analysis. The diazinone preparations used were kindly supplied by Messrs. J. R. Geigy, Basel.

[3] K.-B. AUGUSTINSSON, Meth. biochem. Analysis *5*, 1 (1957).

[4] K.-B. AUGUSTINSSON and G. JONSSON, Acta chem. scand. *11*, 275 (1957).

[5] J. W. COOK, J. Ass. off. agric. Chem. Wash. *37*, 984 (1954).