Unsaturated Lactones and Mercaptans. Part III.[1] Lactam of 2-(β-Carboxyethyl)-2-methylthiazolidine-4-carboxylic acid from α-Angelica Lactone (Pent-3-en-4-olide) and Cysteine, and its N.m.r. Spectrum

By **Nils Hellström,*** **Sven-Olof Almqvist,** and **Margarethe Aamisepp,** Department of Chemistry, Agricultural College of Sweden, Uppsala, Sweden
Sören Rodmar, Institute of Physics, University of Uppsala, Sweden

The reaction product from α-angelica lactone (pent-3-en-4-olide) and cysteine was considered by Cavallito and Haskell to be an eight-membered ring lactam; Black has recently found supporting evidence for this. Reinvestigation by n.m.r., however, reveals the compound to be the lactam of 2-(β-carboxyethyl)-2-methylthiazolidine-4-carboxylic acid. The reaction proceeds in two steps and a mechanism leading to this unexpected structure is given. The identity between this compound and the one from 4-oxopentanoic acid and cysteine is demonstrated.

UNSATURATED lactones exhibit a range of biological activities.[2] In order to elucidate their modes of action, reactions between them and compounds containing amino- and/or mercapto-groups have been studied. Cavallito and Haskell[3] reported the reactions of α- and β-angelica lactones (pent-3- and -2-en-4-olides) with cysteine; they isolated a product in the former case, and suggested an eight-membered lactam ring structure for it. They considered the first step to be an addition of the mercapto-group to the double bond to give a γ-sulphide (see Scheme) but they also mentioned the possibility of lactone ring fission to give an amide. They found a drop in pH to be indicative of reaction of the lactones, but no closer study in this respect was reported.

We found a rapid reaction between these lactones and mercaptoacetic acid at pH 8—11.[1] Addition to the double bond of the β-angelica lactone gave a β-sulphide, but in the case of the α-angelica lactone, thiolysis of the lactone ring gave a thiol ester, which was easily hydrolysed. We therefore expected a thiolysis as the most likely first step in the reaction with cysteine, and remarked that a study was needed to elucidate the reaction steps in this case.

In the meantime, for the reaction of the α-angelica lactone with cysteine Black[4] found evidence for the reaction path as shown in the Scheme and for the structure of the final product suggested by Cavallito and Haskell.[3] Black[4] also found a rise in pH during the reaction, which contradicts the statement of Cavallito and Haskell.[3]

The reaction was studied with L-cysteine and followed by changes in optical rotation and pH. These results are shown in Figures 1 and 2 (conditions in Table 1).

Figure 1 shows the changes in pH. Even with an initial pH of 6 the change in pH is rapid. The final pH values are such as could be expected from the production of an acid group (or the disappearance of a base group) equivalent to the amount of cysteine or lactone used, and the buffering capacity of the reaction mixtures.

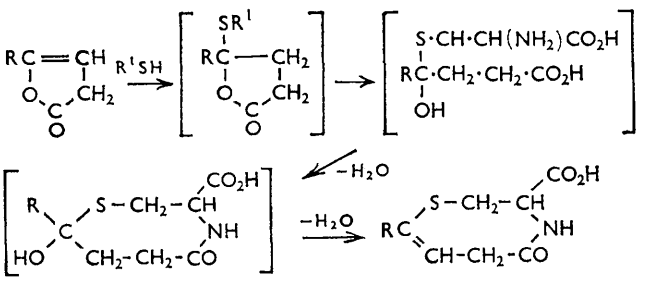
In experiments c and g the change in pH was rapid and the final values were reached after about 15 minutes, but the change in optical rotation was small and slow and final values were not reached during the time interval given in Figure 1. The decrease in optical rotation was more pronounced at low pH. Measurements in experiment f had to be suspended because of crystallisation, but no crystallisation occurred in experiments at higher pH. Acidification of all reaction mixtures to about pH 2 gave the same product (A) (infrared absorption and optical rotation), which had the correct analysis for $C_8H_{11}NO_3S$.

TABLE 1

Reactions of cysteine with α-angelica lactone and with 4-oxopentanoic acid

		a	b	c	d *	e †	f ‡	g	l §	m
Reagent	Cysteine hydrochloride (mmoles) in 15 ml. water	30	30	30	30	30	30	30	30	30
	2N-Sodium hydroxide (mmoles)	60	50	34	34	34	34	28	34	
	α-Angelica lactone (mmoles) in 10 ml. ethanol	30	30	30	30	30	30	30		
	4-Oxopentanoic acid (mmoles)								30	30
Initial pH		9·05	8·10	7·10	7·0	7·10	7·10	6·0	7·2	1·9

* 2N-Sodium hydroxide added after 2·5 hr. to pH 7·5. † pH held constant throughout reaction by addition of 2N-sodium hydroxide. ‡ pH maintained as run e; acidified to pH 1·9 after 1·5 hr. with 3N-hydrochloric acid. § See Experimental section.



SCHEME

[1] N. Hellström, M. Aamisepp, and G. Fuchs, *Lantbrukshögskolans Annaler*, 1964, **30**, 601 (Part I) and 1966, **32**, 427 (Part II).
[2] L. J. Haynes, *Quart. Rev.*, 1948, **2**, 46.
[3] C. J. Cavallito and T. H. Haskell, *J. Amer. Chem. Soc.*, 1945, **67**, 1991.
[4] D. K. Black, *J. Chem. Soc.* (C), 1966, 1123.

To verify that the first step, represented by the rapid decrease in pH (Figure 1), involves production of acid (or consumption of base) sodium hydroxide solution was added continuously to the reaction mixture to keep the pH as constant as possible. Consumption of 0·85 equivalents per mole of lactone or cysteine occurred. findings agree with those of Cavallito and Haskell and of Black [4] and with their postulated structure. However, our n.m.r. evidence supports quite another structure.

Tables 2 and 3 show the resonance frequencies and shifts with assignments in agreement with the integral ratios 1 : 1 : 2 : 4 : 3 (Figure 3). Black,[4] who did not

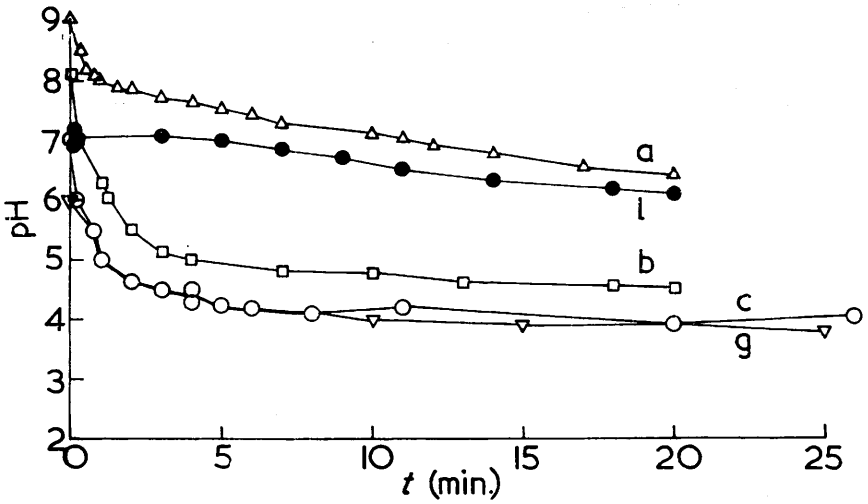


FIGURE 1 Changes in pH of reaction mixtures of cysteine and α-angelica lactone (a, b, c, and g), and cysteine and 4-oxopentanoic acid (l) (see Table 1). The time *t* refers to the moment of mixing the reactants

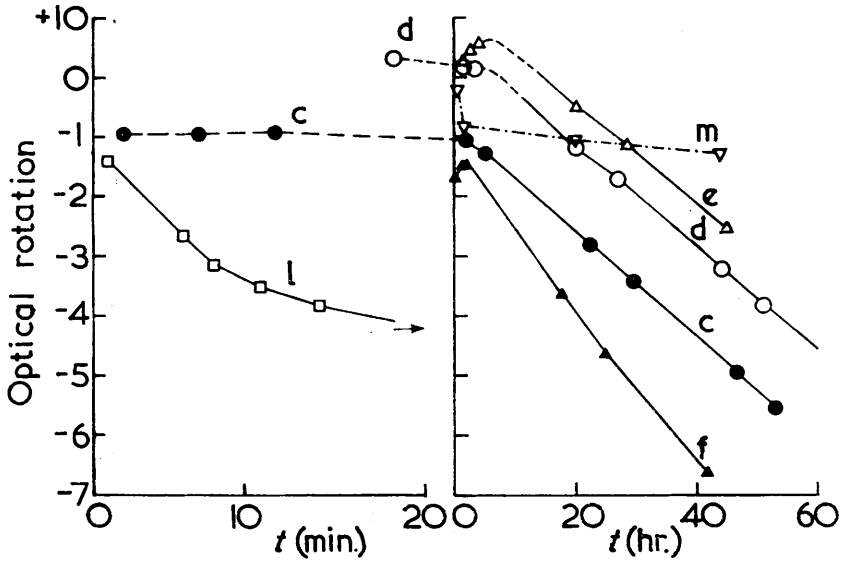


FIGURE 2 Changes in rotatory power of reaction mixtures of cysteine and α-angelica lactone (c, d, e, and f), and cysteine and 4-oxopentanoic acid (l and m) (see Table 1). The time *t* refers to the moment of mixing the reactants, except for d and f, where it refers to the moments given in the footnotes to Table 1

Plots of (total amount alkali — alkali added up to the time $t)^{-1}$ against time gave straight lines, with slopes sensitive to the pH employed.

Compound (A), $C_8H_{11}NO_3S$, contained (pH titration) one carboxy-group. The infrared spectrum showed the presence of a carbonyl and a carboxy-group ($\nu_{max.}$ 1660—1680 and 1740 cm.$^{-1}$), but no conclusive indication (*ca.* 2500 cm.$^{-1}$) of a mercapto-group was found. These refer to any such determinations, quoted peaks at δ 5·48 (two doublets) and 3·84 (four doublets) (ABX; C=CH–CH_2), 2·50 (multiplet; CH–CH_2), and 1·83 (C–Me). These values are all a little higher than ours (δ 5·37, 3·80, 2·44, and 1·80); the difference is about 0·05 units. The fundamental disagreement concerns the assignment of the multiplet with centre at δ 2·44. This prompts some further remarks.

The peak at δ 13·37 is displaced by the addition of water. Dilution also caused displacement with subsequent broadening. This resonance obviously refers to a carboxy-hydrogen; the large down-field shift is possibly due to strong hydrogen-bonding.[5a]

TABLE 2

Line positions and assignments in 56·444 MHz spectrum of the lactam of 2-(β-carboxyethyl)-2-methylthiazolidine-4-carboxylic acid

Transition	Resonance frequency [a]	Chemical shift [b]	Assignment
1	767·40	δ = 13·60	CO_2H
2	312·63		
3	306·78	δ_X = 5·37	X part of ABX system ($CH{\cdot}CH_2$)
4	304·20		
5	298·21		
6	231·59		
7	225·63		
8	220·04	δ_A = 3·91	AB part of ABX system ($CH{\cdot}CH_2$)
9	216·34		
10	214·09	δ_B = 3·70	
11	207·85		
12	204·82		
13	196·29		
14—40	See Table 3	δ_A = 2·73, δ_B = 2·54, δ_C = 2·39, δ_D = 2·19	ABCD system ($CH_2{\cdot}CH_2$)
41	101·49	δ_{Me} = 1·80	CH_3

[a] In Hz (internal tetramethylsilane reference). [b] In p.p.m. (internal tetramethylsilane reference).

Black assigned the peaks at δ 5·37 to a hydrogen on a double bonded carbon atom, but in this region signals for α-hydrogen in α-amino acids are also found.[6,7] With trifluoroacetic acid as solvent the quartet at δ 5·37 turned into a triplet and the shift changed to δ 5·13, and the octet at δ 3·80 turned into a doublet but the shift was the same. The measured splitting was 7·7 Hz.

'Tickling' with irradiation of each of the four lines with centre at δ 5·37 caused splitting of each line in the octet with centre at δ 3·80; in no case was any splitting in the multiplet with centre at δ 2·44 observed. This shows coupling between the quartet and the octet;[8] the former is the X part and the latter the AB part of an ABX system. The following parameters were obtained by an ABX analysis:[5b]

From the AB part:

$$|J_{AB}| = 11{\cdot}54 \text{ Hz} \quad 1/2|J_{AX} + J_{BX}| = 7{\cdot}23 \text{ Hz}$$
$$2D_+ = 15{\cdot}26 \text{ Hz} \quad 2D_- = 17{\cdot}78 \text{ Hz}$$
$$2|D_+ - D_-| = 2{\cdot}53 \text{ Hz}$$

From the X part: $|J_{AX} + J_{BX}| = 14{\cdot}42$ Hz

$$2|D_+ - D_-| = 2{\cdot}58 \text{ Hz}$$

Therefrom:

$$|J_{AB}| = 11{\cdot}54 \text{ Hz} \quad |J_{AX}| = 5{\cdot}47 \text{ Hz} \quad |J_{BX}| = 9{\cdot}00 \text{ Hz}$$
$$\nu_A = 220{\cdot}45 \text{ Hz} \quad \nu_B = 208{\cdot}71 \text{ Hz} \quad \nu_X = 303{\cdot}61 \text{ Hz}$$
$$\delta_A = 3{\cdot}91 \text{ p.p.m.} \quad \delta_B = 3{\cdot}70 \text{ p.p.m.} \quad \delta_X = 5{\cdot}41 \text{ p.p.m.}$$

[5] J. W. Emsley, J. Feeney, and L. H. Sutcliffe, 'High Resolution Nuclear Magnetic Resonance Spectroscopy,' Pergamon, London, (*a*) 1966, vol. 2, pp. 816 *et seq.*; (*b*) 1965, vol. 1, pp. 357 *et seq.*

TABLE 3

Transitions assigned to the ABCD system (Table 2 transitions 14—40, separately recorded and numbered 1—27).

Transition number	Observed position (in Hz from internal tetramethylsilane)	Assignment (in accordance with Figure 5a)	Calculated position from ABCD solution	'Tickling' response on lines [a]
1	181·16	K17	181·17	8R, 9, 17, 18R
2	176·01	A5	176·02	9R, 10R, 15, 17, 25R
3	171·15	A7	171·23	
4	170·05	K4	170·60	
		A6	170·14	
5	165·38	^{13}C-Satellite from Me ?		
6	161·50	A1	161·47	13, 15R, 17R, 19, 22b, 26
7	160·06	A8	160·00	14R, 23
8	156·53	A3	156·49	1, 17, 18R, 19R, 21
9	154·63	C2	154·64	1R, 2R, 6, 19R
		B5	154·64	
		K7	153·63	
		B6	153·00	
10	151·36	K9	151·34	17, 19R, 20R, 25
11	147·66	A2	147·87	3R, 19, 20R,
		B7	147·45	21R, 22b, 23
		K1	147·24	
12	146·18	K18	146·23	4R, 17, 20, 22, 24
13	144·37	K15	144·94	3R, 4, 7, 20,
		C4	144·68	22R, 26
		C6	144·31	
14	143·13	B8	143·24	
15	140·17	D2	140·11	1R, 6, 8, 22b,
		B1	140·09	24
16	137·53	A4	137·54	
17	134·99	K19	134·96	2R, 6, 8, 13, 14, 22bR, 25
		C1	134·95	
		C8	134·54	
18	132·81	B3	132·71	8R, 12
19	129·93	B2	130·73	4, 6, 8R, 9R,
		C5	129·97	11, 12, 24R,
		K8	129·84	26R
20	128·10	K13	128·19	7, 10R, 11R
		D4	128·17	
		C3	127·56	
		K12	127·55	
21	122·77	C7	122·77	1, 2, 3, 11R
22	121·34	D6	121·16	
		B4	120·78	
22b		D1	120·42	
23	116·90	D8	116·95	7R, 14R
		K5	116·20	
24	113·52	K20	113·58	9, 17, 19R
25	111·00	D3	111·05	
26	106·81	D5	106·81	
27	104·95	D7	105·17	

[a] An R after the transition number indicates that the connection with the irradiated transition was found to be regressive (see ref. 8, p. 27 and pp. 167 *et seq.*).

The analysis shows that the couplings J_{AX} and J_{BX} are of the same sign. An INDOR experiment performed by Dr. Attilo Melera, Varian Associates, Zürich, showed

[6] F. A. Bovey and G. V. D. Tiers, *J. Amer. Chem. Soc.*, 1959, **81**, 2870.

[7] M. Hesse, W. V. Philipsborn, D. Schumann, G. Spiteller, M. Spiteller-Friedmann, W. I. Taylor, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, 1964, **47**, 878.

[8] R. A. Hoffman and S. Forsén, 'Progress in Nuclear Magnetic Resonance Spectroscopy,' ed. J. W. Emsley, J. Feeney, and L. H. Sutcliffe, Pergamon, London, 1966, vol. 1, ch. 2.

that J_{AX} and J_{BX} are of the same sign and of opposite sign to J_{AB} (ref. 8, p. 157).

The J values indicate a $CH-CH_2$ grouping. The J_{AB}, J_{AX}, and J_{BX} values are in good agreement with those for ring protons adjacent to a thioether sulphur atom, as in 2,3-dihydrobenzo[*b*]thiophen-3-carboxylic acid where $|J_{AB}| = 12{\cdot}0$, $|J_{AX}| = 5{\cdot}3$, and $|J_{BX}| = 9{\cdot}2$.[9] The J_{AX} and J_{BX} values indicate dihedral angles of about 0 and 120°,[10] *i.e.*, the group is a part of a rigid system as in a smaller ring.

The multiplet with centre at δ 2·44 is shown in detail in Figure 4, and the observed line positions are given in Table 3.

As the integral ratios showed the presence of four protons and as the spectrum was strongly coupled and showed no symmetry, the multiplet was identified as an ABCD subspectrum. The analysis was carried out in the usual way [11] (see also ref. 5, pp. 451 *et seq.*). First from reasonable parameters (shifts and coupling constants) some fifty theoretical spectra were calculated by numerical diagonalisation of the ABCD Hamiltonian matrix on a CDC 3600 computer. The calculated spectra were then compared with the experimental one. About twenty of the experimental lines could then be assigned to appropriate transitions between energy levels in the energy level diagrams [shown in Figures 5(a) and (b)].

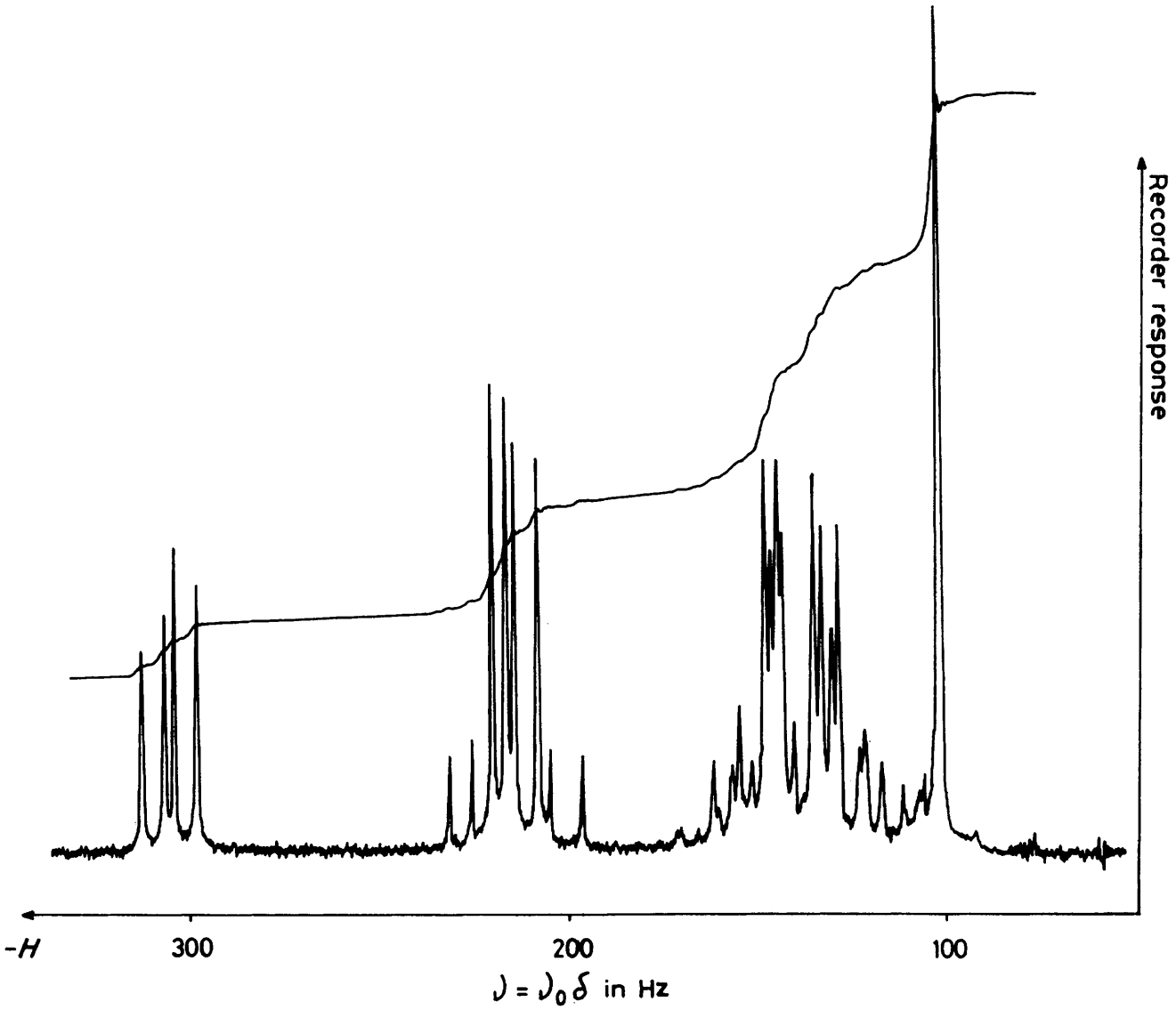


FIGURE 3 N.m.r. spectrum (56·444 MHz) of the lactam of 2-(β-carboxyethyl)-2-methylthiazolidine-4-carboxylic acid with integral curve. The carboxy-resonance at 767·40 Hz is omitted. The methyl resonance is recorded with decreased recorder gain

[9] Ernst Jonsson, private communication.
[10] K. B. Wiberg, 'Physical Organic Chemistry,' Wiley, New York, 1964, (*a*) p. 208; (*b*) pp. 391, 1008.
[11] B. Dischler, *Angew. Chem.*, 1966, **78**, 653.

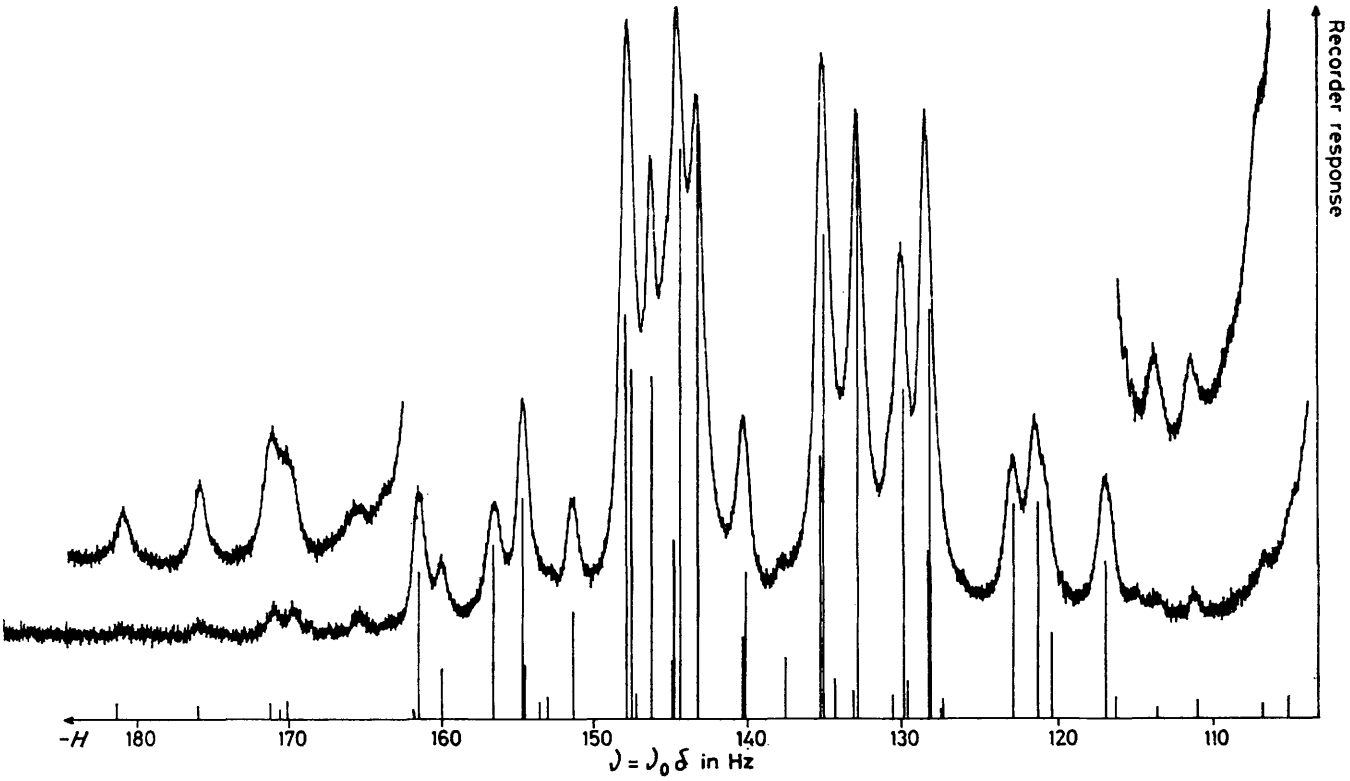


FIGURE 4 N.m.r. spectrum (56·444 MHz) spectrum of the ABCD part with theoretical spectrum. The smaller peaks are recorded with tenfold increase of the sweep frequency field setting

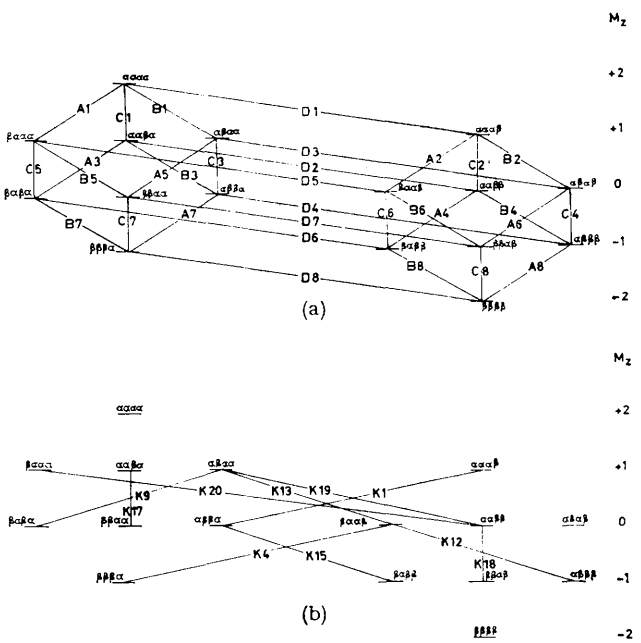


FIGURE 5 Energy level diagram for ABCD n.m.r. spectrum. The symbols of the different transitions have been chosen to correspond with those used in the iteration programme (a) ABCD transitions; (b) combination transitions. Only combination transitions of relevant intensities in the theoretical spectrum have been included

To support and facilitate the assignment, more than twenty 'tickling' experiments were performed (reported in Table 3). The rule of repeated spacings in the spectrum [12] was used in the assignment.

With the starting values of the parameters obtained as above, an iterating procedure was used to get the best least squares fit between theoretical and experimental lines.

The parameters obtained for best fit of the 56·444 MHz spectrum were:

$\nu_A = 154{\cdot}2$ Hz $\quad \nu_B = 143{\cdot}5$ Hz
$\delta_A = 2{\cdot}73$ p.p.m. $\quad \delta_B = 2{\cdot}54$ p.p.m.
$\nu_C = 134{\cdot}7$ Hz $\quad \nu_D = 123{\cdot}4$ Hz
$\delta_C = 2{\cdot}39$ p.p.m. $\quad \delta_D = 2{\cdot}19$ p.p.m.
$J_{AB} = -17{\cdot}7$ Hz $\quad J_{AC} = 7{\cdot}6$ Hz $\quad J_{AD} = 9{\cdot}1$ Hz
$J_{BC} = 10{\cdot}8$ Hz $\quad J_{BD} = 5{\cdot}3$ Hz $\quad J_{CD} = -14{\cdot}0$ Hz

The theoretical and the experimental spectrum are shown in Figure 4.

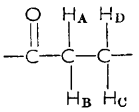
Finally, the theoretical spectrum was recalculated for 100 MHz and compared with one recorded by Dr. Attilo Melera, Varian Associates, Zürich.

Only minor adjustments were needed for complete agreement. The slight change is possibly due to con-

[12] R. A. Hoffman, *J. Pure and Appl. Chem.*, 1965, **11**, 543.

centration dependence of shifts and coupling constants; this, however, was not determined.

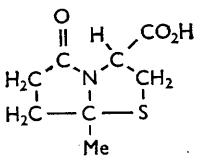
These values indicate a $CH_2 \cdot CH_2$ grouping in a ring system; the geminal coupling constants are −17·7 and −14·0 Hz; the protons A and B are assigned to the methylene group α to the carbonyl group.[13] J_{AD} and J_{BC} are the *cis*, and J_{AC} and J_{BD} the *trans* vicinal coupling constants, hence the grouping is



H_A is less shielded than H_B, whereas H_D, situated *cis* to H_A, is more shielded than H_C.

The values of the vicinal coupling constants are in rough agreement with dihedral angles of 0 and 120° obtained from molecular models.

The n.m.r. study indicates the presence of the fragments CH_3, $CH_2 \cdot CH_2$, and $CH \cdot CH_2$, and leaves no hydrogen for either nitrogen or the sulphur atom. Because of the presence of a carbonyl and a carboxy-group, there must be a quaternary carbon atom. These facts and the nature of the starting materials indicate the probable presence of the structural elements $CH_3 \cdot C \cdot CH_2 \cdot CH_2 \cdot CO$ (from the lactone) and $SCH_2 \cdot CH(N) \cdot CO$ (from cysteine). The only reasonable combination of these seems to be the pyrrolidinothiazolidine structure illustrated.



This bicyclic structure implies two modes of *cis*-annelation, with the lactam ring 'below' or 'above' the thiazolidine ring, *i.e.*, from L-cysteine two diastereoisomers could be formed. However the reaction product seems to be homogeneous. Examination of Leybold models supports the values for the dihedral angles in the $CH_2 \cdot CH$ and the $CH_2 \cdot CH_2$ group indicated by the n.m.r. findings, and also the possibility of hydrogen bonding (in one of the diastereoisomers to the nitrogen atom and in the other more probably to the carbonyl oxygen).

The infrared absorptions $\nu_{max.}$ (dioxan) 1709 and 1715 cm.$^{-1}$, and $\nu_{max.}$ (KBr) 1695 cm.$^{-1}$, have been assigned to substituted fused γ-lactam thiazolidines,[10b,14] and that at 1742 cm.$^{-1}$ (KBr) to a carboxy-group on the thiazolidine ring.[14] Black [4] quoted $\nu_{max.}$ (KBr) 1660—1680 and 1740 cm.$^{-1}$ with the assignments, acid C=O and C=O, respectively. We also found peaks at 1660—1680 and 1740 cm.$^{-1}$. If the assignments made by Black are reversed and the peak at 1695 cm.$^{-1}$ is considered to refer to compounds substituted at the lactam ring, the observed values are not contradictory to the proposed γ-lactam thiazolidine structure.

Oliver *et al.*[15] synthesised this lactam from 4-oxopentanoic acid and cysteine hydrochloride. We repeated this synthesis with L-cysteine and obtained a compound identical with our compound (A). Oliver *et al.* reported that cystine precipitated from the cooled reaction mixture; we also obtained a precipitate, but of cysteine, the most likely product from a reaction mixture containing pyridine.[16] More convenient conditions, similar to those used for some other condensations of ketonic compounds with cysteine and the conversion of other mercapto-amines to thiazolidines,[17] are given in the Experimental section (cf. also Figures 1 and 2).

No evidence for the formation of 4-oxopentanoic acid as an intermediate was found, though an intermediate, (B), $C_8H_{13}NO_4S$, was trapped as its semicarbazone (C) $C_9H_{16}N_4O_4S$.

Cavallito and Haskell [3] found that cysteine containing a blocked mercapto-group did not react with α-angelica lactone (alanine was also unreactive) and they concluded that the γ-sulphide (see Scheme) was an intermediate; however this cannot give a semicarbazone. The first step is therefore, an attack of the mercapto-group, or of the amino-group assisted by the mercapto-group in the form $\bar{S}\overset{+}{R}NH_3$ or $\bar{S}RNH_2$,[18] which causes acyl oxygen fission of the lactone ring and formation of an amide. Intermediate (B) is therefore $Me \cdot CO \cdot [CH_2]_2 \cdot CO \cdot NH \cdot CH(CO_2H) \cdot CH_2 \cdot SH$. In solution, (B) might show ring–chain tautomerism (involving the carbonyl group and either the mercapto-group [19] or the amide group); [20] such a ring tautomer is a reasonable precursor of the final bicyclic product.

EXPERIMENTAL

The n.m.r. spectra were obtained with a Varian HA-60 I Spectrometer at the Institute of Physics, University of Uppsala. The spectrometer was operated at 56·444 MHz in the frequency sweep mode. In the double resonance experiments the r.f. field H_2 with the frequency ν_2 was obtained by amplitude modulation of the magnetic field. The modulation frequency was produced by a Philips PP 6050 oscillator. The audio frequencies were measured with a Hewlett-Packard frequency counter, model 524 B. Solutions in pentadeuteriopyridine with tetramethylsilane (5%) were used. The infrared spectra were obtained with a Perkin-Elmer model 337 spectrophotometer.

α-Angelica Lactone [1] *and Cysteine.*—The reaction mixtures from the experiments shown in Figures 1 and 2 were treated

[13] A. A. Bothner-By in 'Advances in Magnetic Resonance,' ed. J. S. Waugh, Academic Press, New York, 1966, vol. 1, pp. 195 *et seq.*

[14] H. H. Wasserman, B. Suryanarayana, R. C. Koch, and R. L. Tse, *Chem. and Ind.*, 1956, 1022.

[15] G. L. Oliver, J. R. Dann, and J. W. Gates, jun., *J. Amer. Chem. Soc.*, 1958, **80**, 702.

[16] G. Toennies and M. A. Bennett, *J. Biol. Chem.*, 1935—1936, **112**, 497.

[17] V. du Vigneaud and F. H. Carpenter, 'The Chemistry of Penicillin,' ed. H. T. Clarke, J. R. Johnson, and Sir Robert Robinson, Princeton University Press, 1949, p. 1004.

[18] K. Wallenfels and C. H. Streffer, *Biochem. Z.*, 1966, **346**, 119.

[19] G. E. Lienhard and W. P. Jencks, *J. Amer. Chem. Soc.*, 1966, **88**, 3982.

[20] N. H. Cromwell and K. E. Cook, *J. Amer. Chem. Soc.*, 1958, **80**, 4573.

in the following ways. Run a: after 1 day (pH 6·0), 3N-hydrochloric acid (30 mmoles) was added (to pH 1·90). Crystals of the lactam (A) were slowly deposited (26 mmoles after 21 days). Run b; after 1 day (pH 4·0) 3N-hydrochloric acid (22 mmoles) was added (to pH 2·0). Crystals were collected (23 mmoles) which gave the lactam (A), m. p. *ca.* 200° (from water), $[\alpha]_D^{20}$ −227° [Found: C, 47·6; H, 5·6; N, 6·9; S, 15·7%; Equiv. (pH titration), 203. Calc. for $C_8H_{11}NO_3S$: C, 47·8; H, 5·5; N, 7·0; S, 15·9%; Equiv., 201·4].

Reaction mixtures c and g were neutralised to and kept at pH 7·0 for 2 hr.; a stream of oxygen was then passed through. No precipitate (of cystine) was obtained.

In experiments in which the pH was maintained by continuous addition of alkali, after 20, 30, or 40 min. semicarbazide hydrochloride (40 mmoles) was added. Crystals were gradually deposited (10, 15, and 15 mmoles calculated for $C_9H_{16}N_4O_4S$), m. p. 165—168°. Crystals (300 mg.) were dissolved in boiling water and the solution was rapidly cooled to give crystals (120 mg.), of the *semicarbazone* (C), $[\alpha]_D^{20}$ −23° (water) [Found: C, 40·0; H, 6·0; N, 19·1; S, 11·8. $C_9H_{16}N_4O_4S$ requires C, 39·1; H, 5·8; N, 20·3; S, 11·6; 0·9 ($C_9H_{16}N_4O_4S$) + 0·1 ($C_8H_{11}NO_3S$) requires C, 39·8; H, 5·8; N, 19·3; S, 11·9%. When boiled for 5—10 min. in water the compound gave crystals with infrared spectrum identical with that of the lactam (A).

Cysteine and 4-Oxopentanoic Acid.—A reaction mixture such as that in case l (Table 1) was kept under nitrogen for 1 day. The pH fell to 6·25; addition of 2N-sodium hydroxide (4 mmoles) brought the pH to 7·0. Then oxygen was passed through with the pH kept constant by small additions of 2N-sodium hydroxide (in all, 8·2 mmoles). Cystine (2·8 mmoles) precipitated. After precipitation had ceased, the filtrate was acidified with 3N-hydrochloric acid (51 mmoles) to pH 2·0. Solid was deposited during the following 17 days by the slowly evaporating solution, and was recrystallised from water to give a substance (13 mmoles), $[\alpha]_D^{20}$ −223°, identical with the lactam (A) (infrared spectrum).

The lactam of 2-(β-carboxyethyl)-2-methylthiazolidine-4-carboxylic acid, was synthesised by the method of Oliver *et al.*[15] from cysteine hydrochloride and 4-oxopentanoic acid by heating with pyridine in ethanol on a steam-bath for 10 min. The product, $[\alpha]_D^{20}$ −226°, was identical with previous samples of lactam (A) (infrared spectrum). Oliver *et al.*[15] reported that a precipitate of cystine was filtered off after the heating on the steam-bath but they gave no evidence for this statement. We also obtained a precipitate, but identified it as cysteine (infrared spectrum and comparison with authentic material[16]) (Found: C, 29·7; H, 6·0; N, 11·5; S, 26·6. Calc. for $C_3H_7NO_2S$: C, 29·7; H, 5·8; N, 11·6; S, 26·4).

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