SYNTHESIS OF 2-BENZAMIDO-2-MERCAPTOPROPANOIC ACID FROM 4-METHYL-2-PHENYL-2-OXAZOLIN-5-ONE

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Abstract

The structures of the dimers of *N*-substituted pyruvamides are confirmed to be the pyrrolidinones (6). 4-Chloro-4-methyl-2-phenyl-2-oxazolin-5-one reacts with α-toluenethiol to give a thiol ester which can be converted into 2-benzamido-2-benzylthiopropanoic acid. Reaction of the chlorooxazolinone with thioacetic acid gives 4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one which is converted into 2-benzamido-2-mercaptopropanoic acid. The mercapto acid is oxidized by iron(III) chloride–ether to a mixture of disulphides.

Introduction

Gliotoxin[1] (1) and sporidesmin[2] (2) are fungal toxins with interesting antibiotic activity which is apparently related to the presence of a 3,6-epidithiopiperazine-2,5-dione ring system (3) in each structure. Reduction of the disulphide bridge of sporidesmin and alkylation of the resulting bis-thiol destroys the antibiotic activity.[3] The structures of these metabolites were elucidated by chemical means and confirmed by X-ray crystallography.[4,5] Several other toxins having, as their key features, the bridged ring system (3) have since been described.[6]

While our work was in progress, two syntheses of the epidithiopiperazinedione ring system were described.[7,8] Each of them proceeded by halogenation of a symmetrical piperazinedione, replacement of the halogens by a sulphur nucleophile,

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[1] Bell, M. R., Johnson, J. R., Wildi, B. S., and Woodward, R. B., *J. Am. chem. Soc.*, 1958, **80**, 1001.

[2] Hodges, R., Ronaldson, J. W., Shannon, J. S., Taylor, A., and White, E. P., *J. chem. Soc.*, 1964, 26.

[3] Jamieson, W. D., Rahman, R., and Taylor, A., *J. chem. Soc.* (*C*), 1969, 1564.

[4] Fridrichsons, J., and Mathieson, A. McL., *Acta crystallogr.*, 1965, **18**, 1043.

[5] Fridrichsons, J., and Mathieson, A. McL., *Acta crystallogr.*, 1967, **23**, 439.

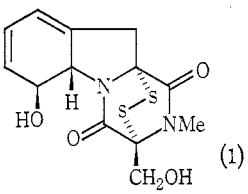
[6] Nagarajan, R., Huckstep, L. L., Lively, D. H., DeLong, D. C., Marsh, M. M., and Neuss, N., *J. Am. chem. Soc.*, 1968, **90**, 2980; Hauser, D., Weber, H. P., and Sigg, H. P., *Helv. chim. Acta*, 1970, **53**, 1061; Taylor, A., "Biochemistry of Some Foodborne Microbial Toxins." p. 69. (Eds R. I. Mateles and G. N. Wogan.) (M.I.T. Press: Cambridge, Mass., 1967.)

[7] Svokos, S. G., and Angier, R. B., Ger. Pat. No. 2029306 (*Chem. Abstr.*, 1971, **74**, 53845); Trown, P. W., *Biochem. biophys. Res. Commun.*, 1968, **33**, 402.

[8] Poisel, H., and Schmidt, U., *Angew. Chem.*, 1971, **83**, 114 and *Chem. Ber.*, 1971, **104**, 1714.

and ultimate formation of the disulphide bridge by oxidation. Finally, coincident with our preliminary communication,[9] there was reported[10] the reaction of the dianion of a symmetrical piperazinedione and sulphur monochloride which gave a low yield of the bridged product (3; R = COOEt, R′ = Me).

In the first two cases the piperazinedione was derived from glycine so that a compound of type (3; R = H) was produced. Since we were more interested in the natural products than in synthetic analogues, we had commenced with the synthesis of fragments of type (4) from which highly substituted unsymmetrical piperazinediones might be synthesized.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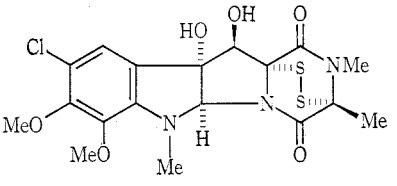
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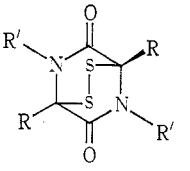
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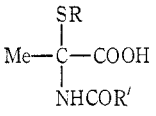
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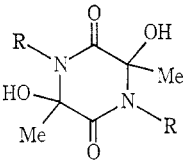
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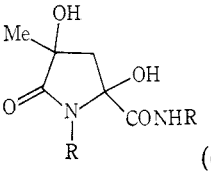
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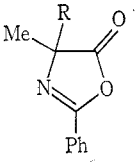
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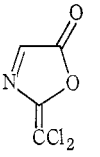
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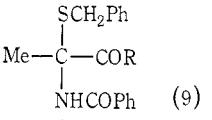
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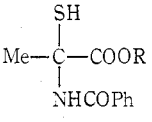
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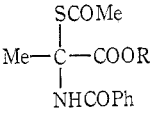
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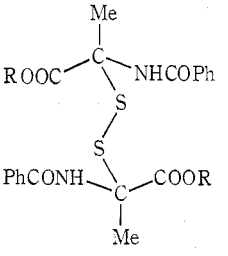
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Pyruvate Reactions

Compounds of type (4) have been reported[11] as products of reaction of pyruvic acid with an acid amide followed by a thiol. In our hands, however, the only product

[9] Pojer, P. M., and Rae, I. D., *Tetrahedron Lett.*, 1971, 3077.
[10] Hino, T., and Sato, T., *Tetrahedron Lett.*, 1971, 3127.
[11] Kaneda, A., and Sudo, R., *Bull. chem. Soc. Japan*, 1970, **43**, 2159.

of such reactions was 2,2-bis(benzylthio)propanoic acid, and the discovery of a number of errors in the original report[11] was enough to destroy our confidence in this route. Next we investigated the condensation of two moles of a pyruvamide which might provide a cyclic structure suitable for the addition of the disulphide bridge. It had been stated[12] that pyruvanilide dimerized to give the piperazinedione (5; R = Ph) but a later report[13] identified the product as the pyrrolidinone (6; R = Ph), largely on the basis of its ready dissociation to pyruvanilide. We have repeated the dimerization in the presence of diethylamine, and confirm the nature of the product as the pyrrolidinone (6; R = Ph). The key observation was the appearance of the methylene proton signals in the proton magnetic resonance spectrum as an AB quartet, and the presence of only one methyl group. Storage of *N*-methylpyruvamide for a year also produced a dimer which could be identified as (6; R = Me) on similar grounds, and also because one of the *N*-methyl signals in the p.m.r. spectrum appeared as a doublet due to H–C–N–H coupling.

2-Benzamido-2-benzylthiopropanoic Acid

Our next strategy was to attempt to add a sulphur-containing substituent to the 2-position of alanine and to this end we explored the chemistry of 4-methyl-2-phenyl-2-oxazolin-5-one (7; R = H). Neither the oxazolinone nor its anion reacted cleanly with α-toluenesulphenyl chloride nor with sulphur monochloride. However, the anion reacted with *S*-benzyl-*p*-toluenethiosulphonate[14] to give a small yield of a new compound which was later identified as 4-benzylthio-4-methyl-2-phenyl-2-oxazolin-5-one (7; R = SCH_2Ph).

Compounds of this type had previously been prepared by two routes which involved, respectively, addition of a thiol to the 2-dichloromethylene-3-oxazolin-5-one (8)[15] and displacement by a sulphur nucleophile of the halogen in a 4-halo oxazolinone (7; R = halogen).[16] We chose to follow the latter procedure although the original authors had reported lower yields with the 4-methyl (alanine) series than those reported for the unsubstituted (glycine) series. When the oxazolinone (7; R = H) was dissolved in 1,2-dichloroethane and treated with chlorine, the chlorination of the 4-position was slow and chlorination of the solvent became competitive. More efficient chlorination was achieved by means of sulphuryl chloride in the same solvent, especially when care was taken to prevent the precipitation of oxazolinone hydrochlorides.[17] The oily chloro compound, which remained after removal of solvent and excess reagents, was characterized only by means of its infrared (1830 cm^{-1}) and its p.m.r. spectrum which showed a singlet resonance at δ 2·05 arising from the methyl protons.

[12] Wohl, A., and Lips, L. H., *Ber. dt. chem. Ges.*, 1907, **40**, 2312.

[13] Scudi, J. V., *J. Am. chem. Soc.*, 1937, **59**, 1403.

[14] Brooker, L. G. S., and Smiles, S., *J. chem. Soc.*, 1926, 1723; Chivers, J. C. A., and Smiles, S., *J. chem. Soc.*, 1928, 697.

[15] Steglich, W., Tanner, H., and Hurnaus, R., *Chem. Ber.*, 1967, **100**, 1824.

[16] Chemyakine, M. M., Tchaman, E. S., Denisova, L. I., Ravdel, G. A., and Rodionow, W. J., *Bull. Soc. chim. Fr.*, 1959, 530.

[17] Weygand, F., Steglich, W., and Tanner, H., *Liebigs Ann.*, 1962, **658**, 128 (esp. footnote p. 138).

The crude chloro compound reacted readily with two molar equivalents of α-toluenethiol to give *S*-benzyl 2-benzamido-2-benzylthiopropanethioate (9; R = SCH_2Ph) in high yield. The ring-opening was competitive with the nucleophilic displacement of the chlorine and any attempt to use less than two moles of thiol resulted in mixtures. Microanalysis of the thiol ester established the formula as $C_{24}H_{23}NO_2S_2$, and the structure was established by spectroscopic data. The infrared spectrum showed the expected N–H stretching vibration at 3241 cm^{-1} and carbonyl absorptions at 1671 cm^{-1} (thiol ester) and 1643 cm^{-1} (amide). The p.m.r. spectrum contained singlet resonances assigned to the methyl and the two benzylic methylene groups. Attempted aminolyses of this thiol ester with a number of aliphatic and aromatic amines were unsuccessful, but it could be smoothly hydrolysed in aqueous sodium hydroxide containing a trace of α-toluenethiol.

The carboxylic acid so obtained (9; R = OH) analysed correctly for $C_{17}H_{17}NO_3S$, and satisfactory spectroscopic data were recorded for it. The only notable feature of these latter was that the benzylic methylene protons gave rise to an AB pattern with doublets centred at δ 3·89 and δ 4·05 (J_{AB} 13·5 Hz) in the p.m.r. spectrum. The methylene protons in such compounds are diastereomeric,[18] because of the adjacent asymmetric centre, and the singlet observed in the spectrum of the thiol ester (9; R = SCH_2Ph) must arise from fortuitous equivalence of chemical shifts. The acid decomposed when treated with thionyl chloride at 0°, but a methyl ester (9; R = OMe) was obtained by reaction of the acid with diazomethane. The ester was resistant to aminolysis, presumably because of steric hindrance by the 2-substituents.

In an attempt to form amides of the acid (9; R = OH) it was converted into the oxazolinone (7; R = SCH_2Ph) by reaction with dicyclohexylcarbodiimide. The formation of the oxazolinone, which was an oil, was confirmed by the appearance of a characteristic carbonyl absorption at 1823 cm^{-1} in the infrared spectrum and an upfield shift of 0·2 p.p.m. in the resonance position of the methyl protons. However, the oxazolinone ring was also resistant to aminolysis and hydrolysis although it reacted with α-toluenethiol, in accord with the initial reaction described above. This low reactivity of 4,4-disubstituted oxazolinones has been noted previously.[19]

The benzylthio group had been introduced in the belief that it would survive most synthetic manipulations, and that the benzyl group could be cleaved from the sulphur at the appropriate time. Thus we turned our attention to this debenzylation, beginning with metal–amine reductions which are commonly used to achieve this cleavage.[20] In the belief that the thiol (10; R = H) would be somewhat sensitive, we chose an oxidative workup for these reactions, hoping to isolate a more stable disulphide. The product, however, was invariably benzyl disulphide whose formation betrays the cleavage of the sulphide in the wrong sense. Perhaps this reflects cleavage

[18] Jackman, L. M., and Sternhell, S., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry." 2nd Edn, p. 371. (Pergamon: Oxford 1969); Mislow, K., "Introduction to Stereochemistry." p. 73. (Benjamin: New York 1965.)

[19] Cornforth, J. W., "The Chemistry of Pencillin." p. 785. (Eds H. T. Clarke *et al.*) (Princeton University Press 1949); Bergamann, M., and Grafe, K., *Hoppe-Seyler's Z. physiol. Chem.*, 1930, **187**, 183.

[20] Smith, Herchel, "Organic Reactions in Liquid Ammonia." p. 191. (Interscience: New York 1963.)

of an intermediate radical anion to give the mercaptide anion and an α-carboxy radical. An alternative debenzylation, which involved oxidation of the sulphide to a sulphoxide, Pummerer rearrangement with formation of an α-acetoxy sulphide, and hydrolysis of this compound under mild conditions also broke down at the first stage. Oxidation of the acid (9; R = OH) under a variety of conditions led to cleavage of the same S–C bond and the isolation of benzyl disulphide or its oxidation products. A final attempt to form an α-chlorosulphide led to rearrangement and the formation of 2-benzamido-3-benzylthioacrylic acid which is described in a separate communication.[21]

2-Benzamido-2-mercaptopropanoic Acid

The difficulties encountered in the removal of the benzyl protecting group led us to the use of the acetyl group in its stead. Thioacetic acid reacted with the chlorooxazolinone (7; R = Cl) to give 4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one (7; R = SCOMe). The formula was established by microanalysis and mass spectrometry, and the infrared spectrum showed carbonyl absorption at 1810 cm^{-1} (oxazolinone ring) and 1697 cm^{-1} (acetylthio). The p.m.r. spectrum showed methyl proton singlets at δ 1·72 and 2·27 and a five-proton multiplet for the aromatic protons.

The mechanistic similarity between the steps required to open the oxazolinone ring and those to remove the acetyl protecting group caused some concern but, in the event, the ring was more reactive. The oxazolinone was dissolved in ether and the solution saturated with dry hydrogen chloride gas. The solvent was removed and the pinkish solid was triturated with water to give, in good yield, the acid (11; R = H). Satisfactory analytical and mass spectrometric data were obtained and the structure was easily confirmed by means of the infrared and p.m.r. spectra. The acid could be esterified with diazomethane to give a crystalline methyl ester (11; R = Me) which had a double melting point. As with the benzylthio acid described above, the acid (11; R = H) was converted into an oxazolinone (7; R = SCOMe) by treatment with dicyclohexylcarbodiimide, and the oxazolinone did not react smoothly on attempted aminolysis.

The acetylthio acid (11; R = H) was hydrolysed to 2-benzamido-2-mercaptopropanoic acid (10; R = H) in 69% yield by means of hydrochloric acid in methanol. The product was obtained as a colourless powder, m.p. 146–147° (with decomposition), which was surprisingly stable towards atmospheric oxidation and which survived mild treatment with aqueous sodium hydroxide. The infrared spectrum confirmed the loss of the *S*-acetyl group but no band could be assigned to the S–H stretching vibration, possibly because this region was obscured by the extended O–H peak of the carboxylic acid. However, the thiol proton was readily visible in the p.m.r. spectrum as a broadened singlet at δ 3·8 which was slowly removed when the sample was shaken with deuterium oxide. The mercapto acid was methylated with diazomethane to give the ester (10; R = Me), identical with that obtained by deacetylation of the acetylthio ester (11; R = Me) described above. The mercapto ester showed a sharp peak in the infrared spectrum at 2580 cm^{-1} which was assigned to the S–H stretching vibration, and a thiol proton resonance at δ 3·62 in the p.m.r. spectrum. Some of this ester could also be isolated from treatment of the acetylthiooxazolinone

[21] Pojer, P. M., and Rae, I. D., *Tetrahedron Lett.*, 1971, 3081.

with neat sulphuryl chloride (8 drops). A vigorous, exothermic reaction ensued with copious evolution of a fuming gas (presumably hydrogen chloride). After the reaction had subsided, the mixture was poured into the main reaction vessel which was removed from the ice bath and thoroughly shaken. The colourless suspension suddenly dissolved, leaving a clear, colourless solution. Sometimes this process had to be repeated before solution occurred. The reaction vessel was replaced in the ice bath and, under stirring, the remainder of the sulphuryl chloride solution was added dropwise over 1 hr. The clear mixture was stirred at 0° for a further 2 hr. The solvent was then evaporated under high vacuum (1 mm) at a temperature not exceeding 20° to yield 4-chloro-4-methyl-2-phenyl-2-oxazolin-5-one (quantitative yield) as a thick, colourless syrup. Infrared spectrum (neat): ν_{max} 1830, 1635, 1598, 1321, 1293, 1177, 1087, 1071, 1011 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 2·05 (CH_3); m, 5, 7·39–8·18 (ArH).

S-*Benzyl 2-Benzamido-2-benzylthiopropanethioate* (*9*; $R = SCH_2Ph$)

A solution of α-toluenethiol (10 g, 81 mmol) in 1,2-dichloroethane (20 ml) was added over a period of 15 min to a stirred solution of freshly prepared, crude 4-chloro-4-methyl-2-phenyl-2-oxazolin-5-one (6 g, 28·6 mmol) in 1,2-dichloroethane (30 ml) at room temperature. The reaction mixture turned reddish brown and was left stirring at room temperature for 48 hr. The solvent and excess α-toluenethiol were removed under high vacuum (1 mm) at 70°. The syrupy product was purified by column chromatography (20 g silica per gram of product). Traces of α-toluenethiol were eluted first with benzene–light petroleum (1 : 3) followed by the yellow, solid product which was eluted with ether. Recrystallization from benzene–light petroleum yielded S-*benzyl 2-benzamido-2-benzylthiopropanethioate* (10·5 g, 88%) as colourless needles, m.p. 88·5–89·5° (Found: C, 68·2; H, 5·4; S, 15·1. $C_{24}H_{23}NO_2S_2$ requires C, 68·4; H, 5·5; S, 15·2%). Infrared spectrum: ν_{max} 3241, 1671, 1643, 1604, 1581, 1309, 1300 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 2·10 (CH_3); s, 2, 3·68 ($CSCH_2$); s, 2, 4·20 ($COSCH_2$); m, 16, 7·0–7·65 (ArH, NH).

2-Benzamido-2-benzylthiopropanoic Acid (*9*; $R = OH$)

S-Benzyl 2-benzamido-2-benzylthiopropanethioate (1·0 g) was dissolved in ethanol (2 ml) and 1M sodium hydroxide (4 ml) was added. α-Toluenethiol (3 drops) was added to the mixture. Omission of α-toluenethiol resulted in low yields of product. The mixture was warmed to 40°, stirred at room temperature for 30 min, and 1M sodium hydroxide (3 ml) added. The resulting precipitate was redissolved by the addition of ethanol (10–20 ml). The solution was stirred for a further 30 min at room temperature, most of the ethanol was removed on a rotary evaporator, and the aqueous mixture was almost neutralized (final pH 8–9) by careful addition of 2M hydrochloric acid. The oily α-toluenethiol was extracted with ether (4 × 30 ml) and the ether layer was discarded. Air was bubbled through the aqueous layer at 30° to evaporate the last traces of ether and the clear solution was then acidified with 10·5M hydrochloric acid. The cream precipitate was collected by filtration, washed with a very small quantity of ether, and recrystallized from ethanol–water to yield *2-benzamido-2-benzylthiopropanoic acid* (500 mg, 67%) as small, colourless cubes, m.p. 132–134° (Found: C, 64·4; H, 5·4; S, 9·8. $C_{17}H_{17}NO_3S$ requires C, 64·7; H, 5·4; S, 10·2%). Infrared spectrum: ν_{max} 3235, 3000–2650 (broad), 1711, 1642, 1327, 1277, 1179, 1113 (sharp), 1072, 1030 (sharp) cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 2·02 (CH_3); ABq, 2, 3·89 and 4·05, J_{AB} 13·5 Hz (CH_2); s, 1, 6·89, slowly removed by D_2O exchange (NH); m, 10, 7·16–7·52 (ArH); s, 1, 8·98 readily removed by D_2O exchange (COOH).

Methyl 2-Benzamido-2-benzylthiopropanoate

2-Benzamido-2-benzylthiopropanoic acid (300 mg) was dissolved in dry methanol (5 ml) and a distilled solution of diazomethane in ether was added until the yellow diazomethane colour persisted. The solvents were evaporated on a steam bath to yield a clear syrup which solidified after several days. The product was recrystallized from methanol–water to yield *methyl 2-benzamido-2-benzylthiopropanoate* (280 mg, 90%) as long, fine colourless needles, m.p. 100·5–101·5° (Found: C, 65·7; H, 5·9; S, 9·6. $C_{18}H_{19}NO_3S$ requires C, 65·7; H, 5·8; S, 9·8%). Infrared spectrum: ν_{max} 3255, 1743, 1640, 1113, 720, 703 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 1·97 (C–CH_3); s, 3, 3·74 (O–CH_3); ABq, 2, 3·78 and 3·92, J_{AB} 14 Hz (CH_2); s 1, 6·83 (NH); m, 10, 7·1–7·5 (ArH).

4-Benzylthio-4-methyl-2-phenyl-2-oxazolin-5-one (*7*; $R = SCH_2Ph$)

2-Benzamido-2-benzylthiopropanoic acid (200 mg, 0·635 mmol) was dissolved in dry tetrahydrofuran (1·5 ml) and a solution of dicyclohexylcarbodiimide (130 mg, 0·635 mmol) in tetrahydrofuran (1 ml) was added at room temperature. The mixture was stoppered, shaken, and left at room temperature for 2 hr. The precipitated dicyclohexylurea (120 mg, m.p. 224° (lit.[24] 226°)) was removed by filtration and the tetrahydrofuran was evaporated under vacuum (14 mm). The crude product was purified by preparative layer chromatography and then distillation to yield *4-benzylthio-4-methyl-2-phenyl-2-oxazolin-5-one* (180 mg, 95%) as a colourless oil, b.p. 140°/0·1 mm (Found: S, 10·8%). No satisfactory microanalytical data could be obtained, and the compound did not show a molecular ion in its mass spectrum. Infrared spectrum (neat): ν_{max} 3300 (small), 1823, 1643, 1602, 1581, 1294, 1158, 1096, 1075, 1009 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 1·79 (CH_3); s, 2, 3·78 (CH_2); m, 10, 7·05–8·19 (ArH).

Reaction of Lithium in Liquid Ammonia with 2-Benzamido-2-benzylthiopropanoic Acid

To stirred, distilled liquid ammonia was added 2-benzamido-2-benzylthiopropanoic acid (500 mg, 1·6 mmol) dissolved in ice-cold tetrahydrofuran (10 ml). Lithium wire (45 mg, 6·4 mmol) was then added to the solution in small pieces over 15 min. The mixture was stirred for 5 min, the ammonia was allowed to evaporate, and a solution of iodine in methanol was added until the iodine colour persisted. The reaction mixture was acidified with 2N hydrochloric acid, 5% aqueous sodium metabisulphite solution (10 ml) was added, and the organic solvents were partly evaporated under vacuum (14 mm). The remaining aqueous mixture was extracted with ether (3 × 30 ml) which was dried over anhydrous sodium sulphate and evaporated. The brown, syrupy residue solidified and was recrystallized from ethanol–water to yield dibenzyl disulphide (90 mg, 46%), m.p. and mixed m.p. 65°. The infrared and p.m.r. spectra were identical with those of an authentic sample.

Attempted Sulphoxidation of 2-Benzamido-2-benzylthiopropanoic Acid

(i) 2-Benzamido-2-benzylthiopropanoic acid (500 mg, 1·6 mmol) was suspended in a mixture of glacial acetic acid (2 ml) and acetic anhydride (4 drops). The mixture was cooled to 15° and 30% hydrogen peroxide (0·25 ml, 4 mmol) was added dropwise. The suspension gradually dissolved but after 30 min a fine, colourless precipitate formed. Ice (1 g) was added and the solid product was collected by filtration. Recrystallization from ethanol yielded dibenzyl disulphide (117 mg, 60%), m.p. 68°. The infrared spectrum was identical with that of an authentic sample.

The aqueous filtrate obtained after removal of the dibenzyl disulphide was treated with *o*-phenylenediamine as a test for pyruvic acid.[25] The test was negative.

When a solution of 2-benzamido-2-benzylthiopropanoic acid in acetone was treated with hydrogen peroxide at room temperature for 15 hr, the isolated product was *S*-benzyl α-toluenethiosulphonate, m.p. 108°. The infrared spectrum was identical with that of an authentic sample.

(ii) A solution of 2-benzamido-2-benzylthiopropanoic acid (300 mg, 0·95 mmol) in glacial acetic acid (2 ml) was added to a solution of (diacetoxyiodo)benzene (300 mg, 0·95 mmol). The mixture was left at room temperature for 30 min and then refluxed for 2 hr. The dark brown solution was poured onto crushed ice (10 g). The precipitate was collected and recrystallized from ethanol to yield dibenzyl disulphide, m.p. 68°. The infrared spectrum was identical with that of an authentic sample.

Dibenzyl disulphide was also isolated when a solution of 2-benzamido-2-benzylthiopropanoic acid (300 mg, 0·95 mmol) in benzene (10 ml) was treated with (diacetoxyiodo)benzens (300 mg, 0·95 mmol) at room temperature for 18 hr.

4-Acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one (*7*; $R = SCOMe$)

4-Chloro-4-methyl-2-phenyl-2-oxazolin-5-one was prepared from 4-methyl-2-phenyl-2-oxazolin-5-one and sulphuryl chloride as described earlier.

An ice-cold solution of freshly prepared 4-chloro-4-methyl-2-phenyl-2-oxazolin-5-one (6 g, 28·6 mmol) in 1,2-dichloroethane (20 ml) was treated, over 5 min, with thioacetic acid (4 g,

[24] Dekker, C. A., and Khorana, H. G., *J. Am. chem. Soc.*, 1954, **76**, 3522.
[25] Hoffman, N. E., and Killinger, T. A., *Analyt. Chem.*, 1969, **41**, 162.

52·5 mmol) in 1,2-dichloroethane (6 ml). The yellow solution was stirred at 0° for 5 min (longer reaction times led to decreased yields) and the solvent and excess thioacetic acid were evaporated at 0° under high vacuum (1 mm). The residue, a thick yellow syrup, was triturated with cold ethanol (3 ml). The solid product which formed was collected by filtration, washed with a little cold ethanol, and recrystallized from ethanol (charcoal) to yield *4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one* (5·3 g, 75%) as colourless, microscopic needles, m.p. 116·5–118° (Found: C, 57·8; H, 4·6; N, 5·8; S, 12·6. $C_{12}H_{11}NO_3S$ requires C, 57·8; H, 4·4; N, 5·6; S, 12·8%). Infrared spectrum: ν_{max} 1825, 1810, 1697, 1638, 1597, 1577, 1320, 1306, 1293, 1153, 1016 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 1·72 (C–CH_3); s, 3, 2·27 ($COCH_3$); m, 5, 7·32–8·2 (ArH). Mass spectrum (mol. wt 249): *m/e* 251 (0·2%), 250 (0·4%), 249 (1·2%), 207 (1·1%), 174 (16%), 146 (2·2%), *105* (*100*%), 77 (50%).

2-Acetylthio-2-benzamidopropanoic Acid

4-Acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one (50 mg) was dissolved in dry ether (3 ml) and dry hydrogen chloride was bubbled through the solution which was immersed in a water bath maintained at about 20°. When the solution was fully saturated, the ether was evaporated on a steam bath to yield a reddish, fuming product which was quickly hydrolysed by moist air to a cream solid substance. Recrystallization of this product from benzene yielded *2-acetylthio-2-benzamidopropanoic acid* (50 mg, 95%) as a colourless powder, m.p. 129–130° (Found: C, 54·2; H, 5·0; S, 11·8. $C_{12}H_{13}NO_4S$ requires C, 54·0; H, 4·9; S, 12·0%). Infrared spectrum: ν_{max} 3305, 2650–2550 (broad), 1721 (shoulder), 1680, 1664, 1205, 1108, 1078 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 2·17 (C–CH_3); s, 3, 2·30 ($COCH_3$); m, 6, 7·3–8·1 (ArH, NH); s, 1, 9·87, readily removed by D_2O exchange (COOH). Mass spectrum (mol. wt 267): *m/e* 192 (1%), 191 (2%), 147 (1%), 146 (1%), 141 (2%), 105 (60%), *77* (*100*%).

Methyl 2-Acetylthio-2-benzamidopropanoate

2-Acetylthio-2-benzamidopropanoic acid (200 mg) was dissolved in a mixture of ether (5 ml) and methanol (2 ml) and a distilled solution of diazomethane in ether was added until the yellow diazomethane colour persisted. The solvent was evaporated on a steam bath to yield a colourless syrup which solidified over a period of 2 weeks. The product was recrystallized from light petroleum–benzene to yield *methyl 2-acetylthio-2-benzamidopropanoate* (190 mg, 90%) as rosettes of colourless needles, m.p. 111–113° and again 121–123° (Found: C, 55·7; H, 5·5; S, 11·0. $C_{13}H_{15}NO_4S$ requires C, 55·7; H, 5·4; S, 11·4%). Infrared spectrum: ν_{max} 3380, 1740, 1681, 1644, 1105, 1082 cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 2·08 (C–CH_3); s, 3, 2·23 ($COCH_3$); s, 3, 3·85 (O–CH_3); m, 6, 7·28–8·00 (ArH, NH). Mass spectrum (mol. wt 281 (very small)): 206 (4%), 180 (2%), 178 (2%), *105* (*100*%), 77 (93%) with a metastable peak at *m/e* 56·5.

Preparation of 4-Acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one from 2-Acetylthio-2-benzamidopropanoic Acid

To a solution of 2-acetylthio-2-benzamidopropanoic acid (100 mg, 0·37 mmol) in dry tetrahydrofuran (2 ml) was added a solution of dicyclohexylcarbodiimide (78 mg, 0·37 mmol) in dry tetrahydrofuran (1·5 ml). The mixture was stoppered, shaken, and left at room temperature for 2 hr. The precipitated dicyclohexylurea (79 mg, m.p. 225° (lit.[24] 226°)) was removed by filtration and the tetrahydrofuran was evaporated on a boiling water bath. The oily residue quickly solidified to yield, after recrystallization from ethanol, colourless microcrystals of 4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one (95 mg, 95%), m.p. 116°. Infrared spectrum: ν_{max} 1825, 1810, 1695, 1638, 1597 cm^{-1}.

2-Benzamido-2-mercaptopropanoic Acid

Aqueous 10·5M hydrochloric acid (20 ml) was added dropwise over 15 min to a vigorously stirred solution of 2-acetylthio-2-benzamidopropanoic acid (5 g) in methanol (25 ml) at 20°. A gentle stream of air was played on the stirred mixture and when half the solvent had evaporated, 5·5M hydrochloric acid (10 ml) was added dropwise, followed by sufficient methanol to dissolve any precipitate. The solvent was completely evaporated by a slow stream of air. This process required about 18 hr at room temperature. The semi-solid residue was triturated with dichloro-

methane (3 ml) and the colourless solid material was collected by filtration. The dichloromethane mother liquor contained small quantities of a second crop of the same substance. Recrystallization of the crude product from chloroform yielded *2-benzamido-2-mercaptopropanoic acid* (2·9 g, 69%) as a colourless powder, m.p. 146–147° (dec.) (Found: C, 53·2; H, 4·9; S, 14·2. $C_{10}H_{11}NO_3S$ requires C, 53·2; H, 4·9; S, 14·2%). Infrared spectrum: ν_{max} 3300, 2600 (broad), 1725, 1620 (broad), 1114, 1074 cm^{-1}. P.m.r. spectrum (DMSO-d_6): s, 3, 1·88 (CH_3); s(b), 1, 3·8, slowly removed by D_2O exchange (SH); m, 6, 7·4–8·1, slow D_2O exchange leaves 5 protons (ArH, NH); s, 1, 9·02, readily removed by D_2O exchange (COOH). Mass spectrum (mol. wt 225): *m/e* 225 (0·2%), 192 (5%), 191 (5%), 121 (10%), *105* (*100*%), 77 (27%), 50 (30%) with metastable peaks at *m/e* 157, 112, 56·5. The pure product travelled as a single substance on silica gel thin-layer chromatography but did not bleach the visualizing agent, iodine.[26]

Methyl 2-Benzamido-2-mercaptopropanoate (*10; R = Me*)

(i) *From methyl 2-acetylthio-2-benzamidopropanoate* (*11; R = Me*).—To an ice-cold solution of methyl 2-acetylthio-2-benzamidopropanoate (300 mg) in dry methanol (4·5 ml) was added saturated methanolic hydrogen chloride (1·5 ml). The mixture was left at 0° for 8 hr and the solvent was evaporated at that temperature under vacuum (1 mm). The syrup residue was triturated with a mixture of dry ether (0·5 ml) and pentane (0·2 ml) and the cream solid substance was collected by filtration. The product was recrystallized from ether–pentane (from 34° to 0°) to yield *methyl 2-benzamido-2-mercaptopropanoate* (185 mg, 73%) as rosettes of thick, shiny needles, m.p. 84–85° (Found: mass of molecular ion: *m/e* 239·061932. $C_{11}H_{13}NO_3S$ requires *m/e* 239·061610). Infrared spectrum: ν_{max} 3245, 2580 (sharp, weak), 1748, 1638, 1611, 1587, 1325, 1310, 1272 (broad) cm^{-1}. P.m.r. spectrum ($CDCl_3$): s, 3, 2·03 (C–CH_3); s, 1, 3·62, slowly removed by D_2O exchange (SH); s, 3, 3·85 (O–CH_3); m, 6, 7·22–7·93, slow D_2O exchange leaves 5 protons (ArH, NH). Mass spectrum (mol. wt 239): *m/e* 239 (2%), 206 (20%), 180 (7%), *105* (*100*%), 77 (33%). The pure product travelled as a single substance on silica gel thin-layer chromatography and bleached the visualizing agent, iodine.[26]

Subsequent methanolyses, on a larger scale, produced syrups which were essentially pure methyl 2-benzamido-2-mercaptopropanoate, as shown by thin-layer chromatography and by the p.m.r. spectrum of the product. However, a solid product could only be obtained after purification by silica gel column chromatography. In these cases varying amounts of benzamide were isolated with a corresponding reduction in yield.

(ii) *From 4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one* (*7; R = SCOMe*).—An ice-cold solution of 4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one (5·0 g) in dry methanol (50 ml) was treated with a saturated methanolic hydrogen chloride solution (25 ml). The mixture was left at 0° for 3 hr and the solvent was evaporated at that temperature under high vacuum (1 mm). The syrup residue was chromatographed on silica gel (50 g) and elution with 2% ether in benzene gave methyl 2-benzamido-2-mercaptopropanoate (1 g, 21%), m.p. 84°. The infrared spectrum was identical with that of an authentic sample.

A high melting fraction (obtained on elution with ether) proved to be benzamide (600 mg, 25%), m.p. and mixed m.p. with an authentic sample 126°.

(iii) *From 2-benzamido-2-mercaptopropanoic acid.*—2-Benzamido-2-mercaptopropanoic acid (50 mg) was dissolved in ether (10 ml) and a distilled solution of diazomethane in ether was added until the yellow diazomethane colour persisted. The solvent was evaporated on a steam bath to yield a colourless syrup which would not solidify. Purification by column chromatography was not attempted. On thin-layer chromatography, the product travelled together with an authentic sample of methyl 2-benzamido-2-mercaptopropanoate and both samples bleached the visualizing agent, iodine.[26] The infrared and p.m.r. spectra of this product were identical with those of an authentic sample of methyl 2-benzamido-2-mercaptopropanoate.

Synthesis of Methyl 2-Benzamido-2-benzylthiopropanoate from Methyl 2-Benzamido-2-mercaptopropanoate

A solution of a mixture of methyl 2-benzamido-2-mercaptopropanoate (100 mg, 0·42 mmol) and benzyl bromide (71 mg, 0·415 mmol) in dry, redistilled acetone (5 ml) was refluxed for 20 hr.

[26] Brown, P. R., and Edwards, J. O., *J. Chromat.*, 1968, **38**, 543.

Thin-layer chromatography of the reaction mixture showed that both reactants were still present and that very little product had formed. Anhydrous potassium carbonate (30 mg, 0·216 mmol) was added and the refluxing was continued. Thin-layer chromatography revealed that the desired reaction was complete in 8 hr. The mixture was cooled, the inorganic materials were removed by filtration and washed with acetone (2 ml). The combined filtrate and washings were evaporated to yield an oily residue which solidified on "seeding" with a small crystal of methyl 2-benzamido-2-benzylthiopropanoate. Recrystallization of the product from methanol–water yielded methyl 2-benzamido-2-benzylthiopropanoate (140 mg, 95%) as colourless needles, m.p. and mixed m.p. 95°. The infrared spectrum was identical with that of an authentic sample.

2,2′-Dibenzamido-2,2′-dithiodipropanoic Acid (12; R = H)

A solution of iron(III) chloride hexahydrate (approximately 10 g) in ether (30 ml) was dried over anhydrous sodium sulphate solution (5 g). The clear yellow supernatant liquid was used in the reaction below. Anhydrous iron(III) chloride was partly insoluble in ether and was unsuitable for the preparation of the above reagent.

A solution of 2-benzamido-2-mercaptopropanoic acid (100 mg) in ether (10 ml) was treated dropwise at room temperature with ethereal iron(III) chloride. The initial intensely blue colour gradually changed to blood red and finally to green. A light green precipitate (presumably iron(II) chloride) had formed, until no further colour change was observed (final colour was greenish yellow) and precipitation of iron(II) chloride was complete. The mixture was washed with 3M hydrochloric acid (5 × 8 ml) until the aqueous layer was colourless and the last traces of iron impurities were extracted with saturated aqueous sodium chloride (2 × 5 ml). The colourless ether solution was dried over anhydrous sodium sulphate and the solvent was evaporated on a water bath. The cream, solid residue was recrystallized from chloroform–methanol to yield the chloroform solvate of *2,2′-dibenzamido-2,2′-dithiodipropanoic acid* (100 mg, 95%) as long, colourless needles, m.p. 161·5–162·5° (Found: C, 48·6; H, 4·1; Cl, 8·1; S, 13·0. $C_{20}H_{20}N_2O_6S_2,\frac{1}{2}CHCl_3$ requires C, 48·5; H, 4·0; Cl, 10·5; S, 12·6%). Recrystallization from benzene–acetone yielded the benzene solvate of *2,2′-dibenzamido-2,2′-dithiodipropanoic acid* as a colourless powder, m.p. 159° (Found: C, 57·0; H, 4·9; S, 12·8. $C_{20}H_{20}N_2O_6S_2,\frac{1}{2}C_6H_6$ requires C, 56·6; H, 4·7; S, 13·1%). Infrared spectrum: ν_{max} 3380 (broad), 2650 (broad), 1720, 1620, 1170, 1074 cm^{-1}. P.m.r. spectrum (DMSO-d_6): 2 × s, 6, 1·78 and 1·87 (CH_3); m, 12, 7·2–8·1, slow D_2O exchange leaves 10 protons (ArH, NH); 2 × s, 2, 8·85 and 9·05, readily removed by D_2O exchange (COOH). Mass spectrum (mol. wt 448): *m/e* 256 (1%), 217 (1%), 191 (8%), 122 (23%), *105* (*100*%), 77 (70%). The analytically pure disulphide (116) travelled as two spots on silica gel thin-layer chromatography but attempts to separate these two fractions on the preparative scale resulted in intractable syrups.

Dimethyl 2,2′-Dibenzamido-2,2′-dithiodipropanoate (12; R = Me)

(i) *From 2,2′-dibenzamido-2,2′-dithiodipropanoic acid (12; R = H).*—2,2′-Dibenzamido-2,2′-dithiodipropanoic acid (200 mg) was dissolved in a mixture of ether (5 ml) and methanol (2 ml) and a distilled solution of diazomethane in ether was added until the yellow diazomethane colour persisted. The solvent was evaporated on a steam bath. The cream, solid residue was recrystallized from light petroleum–dichloromethane to yield *dimethyl 2,2′-dibenzamido-2,2′-dithiodipropanoate* (200 mg, 95%) as colourless microcrystals, m.p. 146–148° (Found: C, 55·4; H, 5·1; S, 13·5. $C_{22}H_{24}N_2O_6S_2$ requires C, 55·5; H, 5·1; S, 13·4%). Infrared spectrum: ν_{max} 3235, 1751, 1739, 1632, 1376, 1104, 1073 cm^{-1}. P.m.r. spectrum ($CDCl_3$): 2 × s, 6, 1·99 and 2·02 (CH_3); 2 × s, 6, 3·81 and 3·92 (O–CH_3); m, 11, 7·02–7·84 (ArH, NH); s, 1, 9·20 (NH). Mass spectrum (mol. wt 476): *m/e* 476 (very small), 271 (2%), 206 (9%), 205 (6%), 180 (2%), 173 (2%), 146 (3%), *105* (*100*%), 77 (52%). Analytically pure dimethyl 2,2′-dibenzamido-2,2′-dithiodipropanoate travelled as two substances on silica gel thin-layer chromatography.

(ii) *From methyl 2-benzamido-2-mercaptopropanoate (10; R = Me).*—A solution of methyl 2-benzamido-2-mercaptopropanoate (100 mg) in ether (10 ml) was treated dropwise at room temperature with ethereal iron(III) chloride (compare procedure for oxidation of 2-benzamido-2-mercaptopropanoic acid). The initial intensely blue colour gradually changed to blood red and

finally to green. A light green precipitate (presumably iron(II) chloride) had formed. Addition of ethereal iron(III) chloride (2 ml in all) was continued until no further colour change was observed (final colour was greenish yellow) and precipitation of iron(II) chloride was complete. The mixture was washed with 3M hydrochloric acid (5 × 8 ml) until the aqueous layer was colourless and the last traces of iron impurities were extracted with saturated aqueous sodium chloride (2 × 5 ml). The colourless ether solution was dried over anhydrous sodium sulphate and the solvent was evaporated on a water bath. The syrupy residue (100 mg) failed to solidify but was identical (thin-layer chromatography and p.m.r. spectrum) with authentic dimethyl 2,2′-dibenzamido-2,2′-dithiodipropanoate obtained by method (i).

Separation of the Diastereomers of Dimethyl 2,2′-Dibenzamido-2,2′-dithiodipropanoate (*12*; *R* = *Me*)

Preparative thin-layer chromatography of dimethyl 2,2′-dibenzamido-2,2′-dithiodipropanoate (100 mg) on silica gel gave, on elution (3 times) with chloroform, two distinct bands (viewed under short wavelength ultraviolet light). Each band was scraped from the plate and extracted with boiling chloroform to give two products, both of which were syrups. P.m.r. spectrum of band of lower R_F value ($CDCl_3$): s, 3, 2·03 (CH_3); s, 3, 3·81 (O–CH_3); m, 6, 7·0–7·9 (ArH, NH). P.m.r. spectrum of the other, larger fraction ($CDCl_3$): s, 3, 2·01 (CH_3); s, 3, 3·96 (O–CH_3); m, 5, 7·1–7·85 (ArH); s, 1, 8·5 (NH). Mass spectrum of the latter diastereomer (mol. wt 476): *m/e* 476 (very small) and prominent peaks at *m/e* 206, 205, 105, 77.

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