The Design and Synthesis of New Triazolo, Pyrazolo-, and Pyridazo-pyridazine Derivatives as Inhibitors of Angiotensin Converting Enzyme

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Bicyclic mimetics of the antihypertensive, angiotensin converting enzyme (A.C.E.) inhibitor, captopril, have been designed with the aid of computer graphics. The synthesis and structure activity relationships of the three bicyclic systems tetrahydro[1,2,4]triazolo[1,2-*a*]-, hexahydropyrazolo[1,2-*a*]- and octahydropyridazo[1,2-*a*]-pyridazinediones are described. The compounds with the terminal carboxy group, the thiol, and the amide carbonyl function orientated most closely to correspond to the three-dimensional array of these groups in bound captopril are the most active inhibitors of anglotensin converting enzyme, *in vitro*.

It has been shown that inhibitors of angiotensin converting enzyme (A.C.E., E.C.3.4.15.1), which transforms the decapeptide angiotensin I to the powerful vasoconstrictor angiotensin II, are promising for the control of high blood pressure. Clinical efficacy has been demonstrated for the nonapeptide SQ 20 881 [1] and the orally active agents captopril (1) [2,3] and MK 421 [enalapril (2)].[4] This investigation has been concerned with utilising existing information on reversible inhibitors of angiotensin converting enzyme to enable the design of compounds with advantage for use *in vivo*.

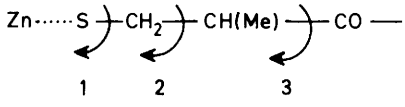
Angiotensin converting enzyme has been isolated from various mammalian sources. It has been well characterised but, as yet, the molecular structure and the nature of the receptor site has not been elucidated by *X*-ray diffraction studies.

The synthetic A.C.E. inhibitors of Ondetti and co-workers [2] were prepared after the elucidation of the structures of natural peptide inhibitors, isolated from snake venoms. Studies on structure-activity relationships [2,5] indicated that derivatives of AlaPro were good substrates for A.C.E., and that some simple analogues retaining *C*-terminal proline were good inhibitors. After drawing the analogy with the mechanism of action and the known structure of zinc-containing carboxypeptidase, it was found that A.C.E., which was also known to contain zinc, incorporated this element in the active site. The compound captopril (1) was designed to bind to zinc through the thiol group, and at the same time, to satisfy the requirement for simulating the binding of AlaPro through other interactions at the active site. Further studies have reinforced the case for regarding the AlaPro moiety as favourable for binding to the A.C.E. active site.[2,4,6] This has resulted in the identification of alternative inhibitors based on this dipeptide.[4,7]

In this investigation we have sought to set out some limits for the co-ordinates for binding on the postulated active centre of A.C.E., using data on captopril as a starting point. In pursuing the design of conformationally restricted analogues of captopril we have assumed that the amide bond remains in the *trans* conformation in the A.C.E.–captopril complex.

This is supported by evidence from the n.m.r. spectrum of captopril in aqueous solution (pH *ca.* 6). From the 400 MHz ^{1}H n.m.r. spectrum and the 75 MHz ^{13}C n.m.r. spectrum (Table 1) the two conformations with *cis* and *trans* amide bonds are clearly observed, but the *trans* form is favoured (Figure 2). The vicinal coupling constants for the SCH_2CH fragment were observed to be 8.7 and 5.7 Hz; this indicated a predominance of rotamer (3a) or (3b) depending on the assignment of the diastereoisotopic protons. The conformation of captopril in the crystal (Figure 1) is very similar to that found in the rotamer (3b); the dihedral angle –S–C–C–(Me) is 71°. In the molecular graphics investigations that follow, this information on the favoured conformation of captopril in the solid state and in solution has been used as the basis for comparison with the stereochemical characteristics of the new synthetic compounds.

Earlier investigations of structure-activity relationships have indicated that binding to the thiol, the terminal carboxy group, and the amide carbonyl is mandatory for high levels of inhibition of captopril analogues.[2,4,7] We have postulated the limits for binding at these three points and plotted them using computer graphics. In the case of the carboxy group, the hoop represented to scale in Figure 9 indicates the limits of interaction with a postulated positively charged centre [NH_3^+ or $NH{\cdot}CH(NH_3^+){\cdot}NH$] of the enzyme. The carbonyl group, which is hydrogen bonded, is regarded as interacting with an –NH– group of the receptor with an optimum angle θ of 150—180° (∠C=O · · · HN). The hydrogen bond donor group of the enzyme is expected to lie within a sector of a sphere of radius *ca.* 1.8 Å from the oxygen atom. The postulated position of the thiol group in relation to the other two functions was derived from the application of computer graphics. In the case of the Zn–thiol interaction it was assumed, initially, that for the array there should be free rotation about



the bonds 2 and 3; on this basis the sulphur atom of captopril traces out the locus derived by the procedure set out in the Experimental section and represented in Figure 3a.

When high energy conformations (>50 kcal/mol †) were excluded, using conventional molecular mechanics calculations,[9] we obtained the mesh plot in Figure 3b. It accommodates the closely related conformations of captopril in the solid state and in solution.

We have chosen the hexahydropyridazine system as a convenient basis for producing variations related to captopril but involving bicyclic systems. Our earlier studies on piperazic acid derivatives [10] had established that there were predictable changes in conformation associated with functionalisation of

† 1 kcal = 4.184 kJ.

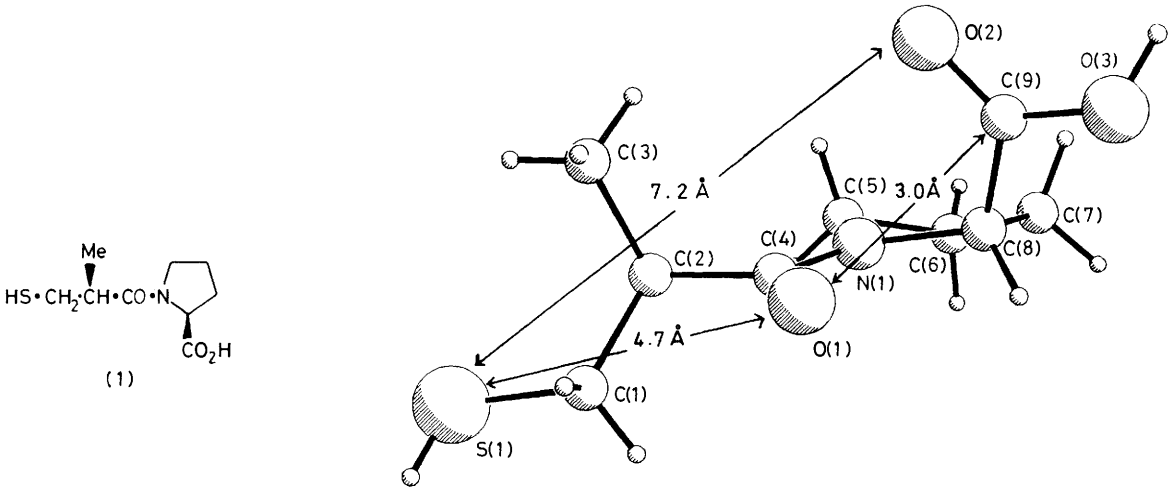


Figure 1. A PLUTO representation of the crystal structure of captopril (from Fujinaga and James [8])

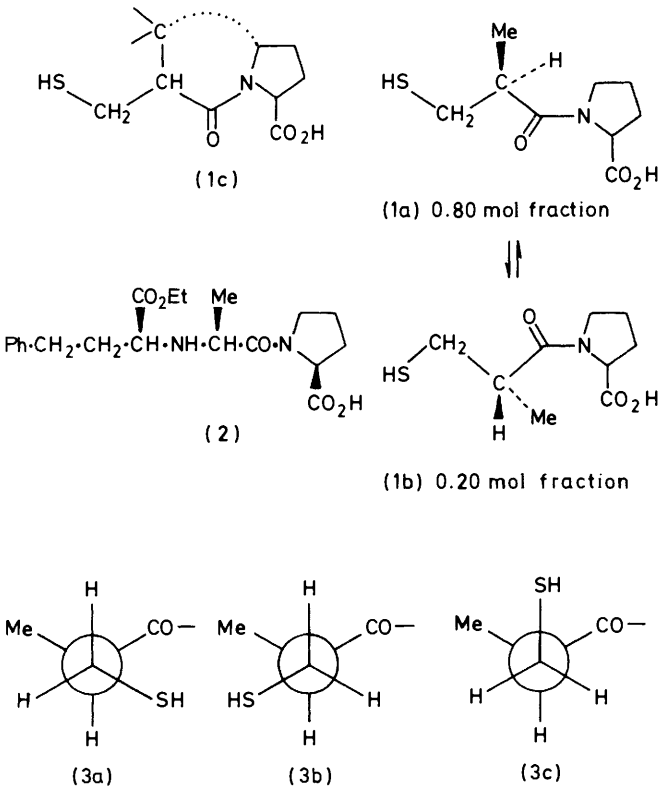


Figure 2. The *trans*- (1a) and *cis*- (1b) isomers of captopril (1c) showing the link required to constrain the molecule in the *trans* form. (3a—c) represent the rotamers about the CH_2–CH(Me) bond in captopril

this system. It provided a basis for obtaining compounds with appropriate variations in the array of the three binding functions.

The three bicyclic systems tetrahydro[1,2,4]triazolo[1,2-*a*]- (4), (5), and (6), hexahydropyrazolo[1,2-*a*]- (7), (8), and (9), and octahydropyridazo[1,2-*a*]-pyridazinediones (10), (11), and (12) have been prepared. The properties of compounds (4), (7), and (10), and analogues, are summarised in Tables 1—3.

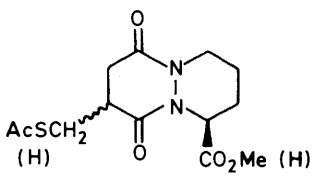
2,3,5,8-Tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]-pyridazine-5-carboxylic Acids (4), (5), and (6).—The bicyclic systems (5) and (6) were readily synthesised by the Diels–Alder reaction of penta-2,4-dienoic acid with the 4-substituted 1,2,4-triazole-3,5-diones (15), prepared from the appropriate isocyanates through the intermediates (13) and (14) as shown in Scheme 1. The red 1,2,4-triazole-3,5-diones (15) were not purified, but were used directly in the Diels–Alder reaction. Related 1,2,4-triazole-3,5-diones are known to be highly reactive dienophiles.[11] The sulphur substituent was introduced by displacement of the chlorine with potassium thioacetate in acetone in the presence of sodium iodide. This reaction was more conveniently carried out with the ester than with the free carboxylic acid, and gave the fully protected bicyclic system (17). Deprotection gave the free thiol carboxylic acids (5) and (6).

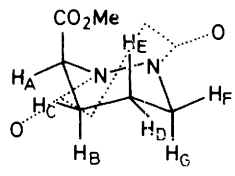
The mercaptomethyl compound (4) was prepared differently. The known [12] triazolopyridazine (18) was esterified and then treated with formaldehyde to give the hydroxymethyl compound (19) (Scheme 2). Treatment with phosphorus pentachloride and potassium thioacetate in turn gave the protected product (15) which hydrolysed to compound (4).

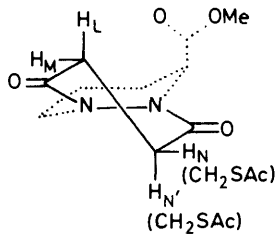
A comparison of models of the [1,2,4]triazolo[1,2-*a*]pyridazine derivatives with captopril suggested that the active form of the derivatives has the *S* configuration at the chiral centre. This was confirmed later by stereospecific synthesis of analogues through (*S*)-piperazic acid. A model for this series of compounds was the dicarboxylic acid (21) (Figure 4) whose structure was determined by *X*-ray diffraction. As predicted by the n.m.r. spectra of these compounds, and others in this series, the right-hand ring adopts an approximate half-chair conformation with the carboxy group pseudoaxially oriented to avoid the 1,3-interaction with the amide carbonyl ('A' strain) [13] The side chain adopts the *trans* (*anti*) conformation as expected. The strain imposed on the molecule by the twisting about the central N–N bond is compensated, to some extent, by a slight deviation of the nitrogen atoms from planarity.

Hexahydro-1,3-dioxo-1H-pyrazolo[1,2-a]pyridazine-5-carboxylic Acids (5), (7), (8), *and* (9).—The bicyclic system (7) might also be synthesised by a Diels–Alder reaction, using the dienophilic reactivity of pyrazole-3,5-diones.[11] However, the failure to prepare the appropriate dienophile readily led us to pursue the compounds using the monoprotected piperazic acid (22) [14] (Scheme 3). Esterification with isobutene gave the

Table 1. N.m.r. analyses for the bicyclic compounds (10), (11), and (12) *







(12),(28),(29)

	Chemical shifts ‡ (δ)		
	(12)	(28)	(29)
A	5.25	5.31	5.32
B	1.67	1.87	1.85
C	2.26	2.41	2.42
D	1.51	1.77	1.74
E	1.32	1.51	1.48
F	4.56	4.67	4.69
G	2.67	3.00	2.78
L	2.87	3.13	2.82
M	2.35	2.56	2.67
N	2.38	2.86	
N′	2.68		2.45
O †		2.91	3.32
P †		3.26	3.05
Q †	1.5	3.74	3.75
R †		2.36	2.35

	Coupling constants (*J*) §		
	(12)	(28)	(29)
AB	5.7	5.9	5.6
AC	2.0	1.7	2.0
AD	1.0	0.6	
BC	−13.5	−13.9	−13.8
BD	4.0	4.2	4.0
BE	13.3	13.6	13.7
CD	2.0	2.9	*ca.* 2.0
CE	3.9	4.1	4.0
CF	2.0	2.0	*ca.* 2.0
CG	0.4	0.5	
DE	−13.0	−13.6	−13.9
DF	2.0	2.4	*ca.* 2.0
DG	3.0	3.3	3.0
EF	4.0	4.2	4.0
EG	13.0	13.1	13.0
FG	−12.9	−12.9	−13.0
LM	−15.0	−16.2	−15.0
LN	4.2	5.0	
LN′	15.0		14.2
MN	2.0	2.2	
MN′	4.5		4.1
NN′	−15.0		
NO		7.3	
N′O			5.7
NP		8.9	
N′P			6.0
OP		−13.8	−13.7

* Compounds (10) and (11) analysed as the *S*-acetyl methyl esters (28) and (29), respectively. (12), unsubstituted on left-hand ring, CO_2H on right-hand ring; (28), side chain *trans* to CO_2Me; (29), side chain *cis* to CO_2Me. † O and P are the two side chain protons CH_2S, Q = OMe [(28) and (29)] or −OH [(12)]. ‡ For $CDCl_3$ solutions, referred to tetramethylsilane. § ±0.1 Hz, confirmed by computer simulation.

corresponding t-butyl ester which was acylated with ethyl methylmalonyl chloride to give the intermediate (23). After catalytic hydrogenolysis of the benzyloxycarbonyl group, cyclisation to the pyrazolopyridazine (24) was achieved by heating in acetic acid. Direct diacylation of the piperazic acid esters with malonyl dichlorides was found to be much less satisfactory.

The appropriate substituents were readily introduced by alkylation of the sodium salt of (24) using *S*-bromomethyl thioacetate [15] and *S*-2-bromoethyl thioacetate [16] to give the fully protected compounds (25). After separation of the diastereoisomers by chromatography, sequential deprotection gave the required thiol acids (7), (8), and (9).

The stereochemistry of the diastereoisomeric pairs and the conformation of the rings was eludicated using physical methods. Prior to obtaining the crystal structure of one of the compounds (9), it was observed that in the ^1H n.m.r. spectra the chemical shifts of the methyl groups in the diastereoisomeric pairs were slightly different in each case; for each pair the shift difference was *ca.* 0.14 p.p.m. It was argued that the methyl group *cis* to the carboxy group, about the mean molecular plane, would be in the deshielding zone of an axial carboxy group, and, therefore, would resonate at lower field than in the corresponding diastereoisomer. This assignment was confirmed by an *X*-ray diffraction study of the crystalline acid (9). It established (Figure 5) that the five-membered ring in this molecule is almost planar. The six-membered ring adopts a chair conformation with the carboxy group axial, as indicated by n.m.r. spectroscopy. The nitrogen atoms, as in the previous series, deviate slightly from planarity. Relevant chemical shifts are recorded in Table 2 (Experimental section).

Octahydro-6,9-dioxopyridazo[1,2-a]*pyridazines* (10), (11), *and* (12).—The bicyclic pyridazo[1,2-*a*]pyridazines were prepared most conveniently by stepwise diacylation of the appropriate piperazic acid derivatives as for compound (7) (Scheme 5). The required acid chloride (21) was prepared from itaconic anhydride as shown in Scheme 4. Alcoholysis of the anhydride occurs regiospecifically,[17] and the resulting monobenzyl itaconate was treated in succession with thioacetic acid (conjugate addition) and phosphorus pentachloride.

Acylation of (*Z*)-piperazic acid methyl ester with the acid chloride (26) gave the expected compound (27) which was separated into individual diastereoisomers. The less polar diastereoisomer was debenzylated and then the resulting compound was cyclized by treatment with phosphorus pentachloride–pyridine to give the bicyclic compound (28).

From earlier studies on acylated piperazic acid derivatives it was expected that the right-hand ring in bicyclic compounds such as (28) would adopt a chair conformation, with the carboxy group axially oriented. The conformation of the left-hand ring in the bicyclic system was suggested by the crystal structure (at −165 °C) of the hexahydropyridazine-

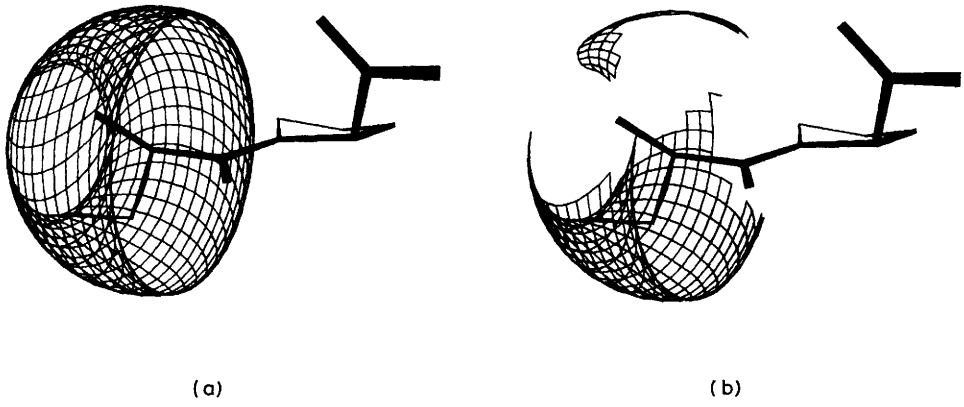
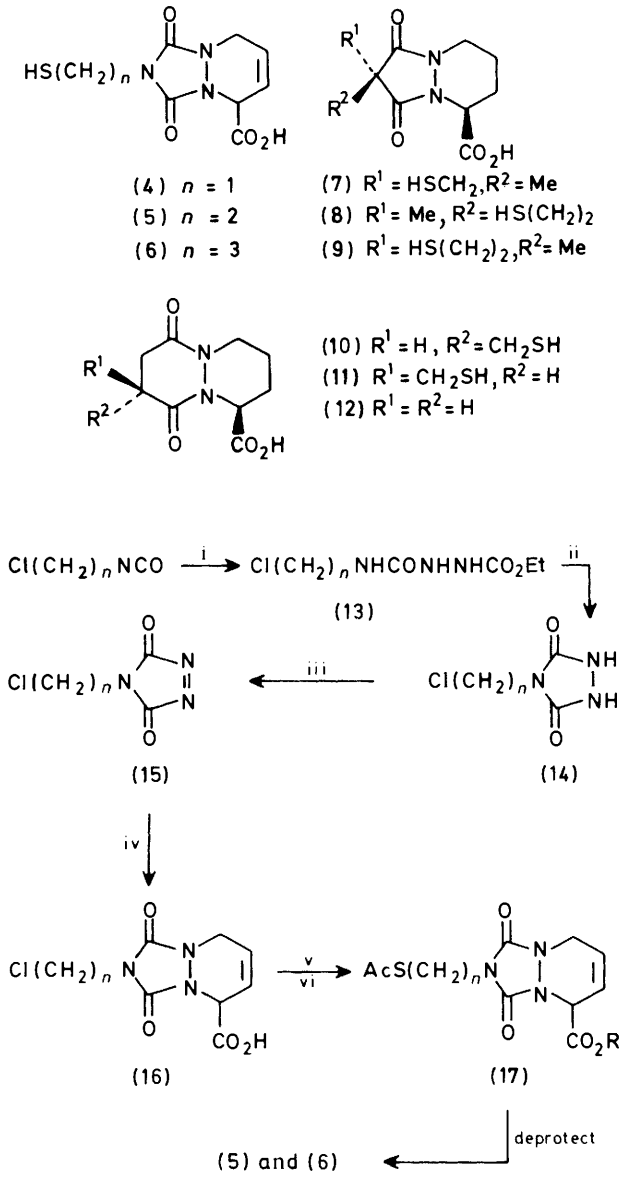


Figure 3. (*a*) The surface mesh (locus) produced for the sulphur atom by allowing free rotation about the CH_2–CH(Me) and CH(Me)–CO bonds; (*b*) the reduced surface after rejecting conformations >50 kcal/mol in total energy



Scheme 1. *Reagents:* i, EtO_2CNHNH_2; ii, 4M-KOH, room temp.; iii, Bu^{t}OCl; iv, penta-2,4-dienoic acid; v, esterification; vi, NaI, acetone, AcSK

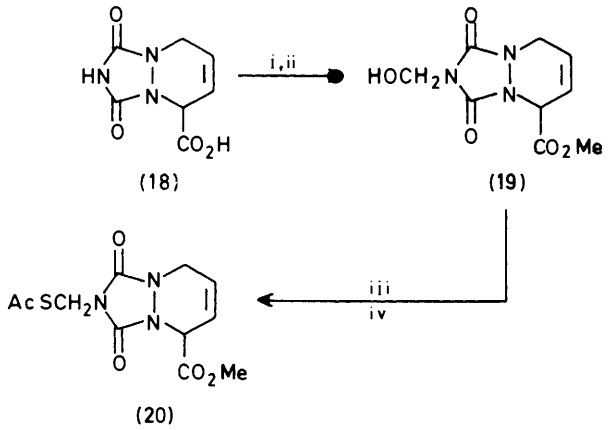
Table 2. N.m.r. data for compounds (4)—(9) and (21)

Compd.	Solvent	δ/p.p.m. (*J* in Hz)
(4)	$CDCl_3$–$(CD_3)_2SO$	2.73 (1 H, t, exch. with D_2O, SH), 4.14 (2 H, m, CH_2C=), 4.66 (2 H, d, s after D_2O exch., CH_2S), 5.00 (1 H, m, $CHCO_2$), 6.10 (3 H, m, 2 H after D_2O exch., CH=CH,CO_2H)
(5)	$CDCl_3$–$(CD_3)_2SO$	1.66 (1 H, t, exch., D_2O, SH), 2.82 (2 H, m, t after D_2O exch., CH_2S), 3.80 (2 H, t, NCH_2CH_2), 4.10 (2 H, m, CH_2C=), 5.10 (1 H, m, $CHCO_2$), 6.10 (2 H, m, CH=CH), 9.2 (1 H, bs, exch., D_2O, CO_2H)
(6)	$CDCl_3$	1.63 (1 H, t, exch., D_2O, SH), 2.02 (2 H, quint., $CH_2CH_2CH_2$), 2.58 (2 H, m, t after D_2O exch., CH_2S), 3.66 (2 H, t, N-CH_2CH_2), 4.15 (2 H, m, CH_2C=C), 5.1 (1 H, m, $CHCO_2$), 6.1, 2 H, m, CH=CH), 7.08 (1 H, bs, exch., D_2O, CO_2H)
(21)	$(CD_3)_2SO$	2.58 (2 H, t, CH_2CO_2H), 3.70 (2 H, t, CH_2CH_2N), 4.0 (2 H, m, CH_2C=C), 5.06 (1 H, m, $CHCO_2H$), 6.06 (2 H, m, HC=CH)
(7)	$CDCl_3$–$(CD_3)_2SO$	1.44 (3 H, s, Me), 1.58 (1 H, t, exch., D_2O, SH), 1.4—2.2 (4 H, m, CH_2-CH_2), 2.82 (2 H, d, s after DO_2 exch.), 3.1 (1 H, m), 4.2 (1 H, m, NCH_2CH_2), 4.9 (1 H, m, $CHCO_2H$), 9.5 (bs, exch., D_2O, CO_2H)
(8)	$CDCl_3$–$(CD_3)_2SO$	1.38 (1 H, t, exch., D_2O, SH), 1.46 (3 H, s, Me), 1.5—2.8 (8 H, m), 3.0 (1 H, m), 4.2 (1 H, m, NCH_2CH_2), 4.9 (1 H, m, $CHCO_2H$), 7.4 (bs, exch., D_2O, CO_2H)
(9)	$CDCl_3$–$(CD_3)_2SO$	1.34 (3 H, s, Me), 1.41 (t, exch., D_2O, SH), 1.6—2.8 (8 H, m), 3.0 (1 H, m), 4.2 (1 H, m, NCH_2CH_2), 4.85 (1 H, m, $CHCO_2H$), 8.3 (bs, exch., D_2O, CO_2H)

dione (29).[18] This established that the compound adopted a twist-boat conformation with C_2 symmetry. It suggested that the octahydro-6,9-dioxopyridazo[1,2-*a*]pyridazines, *e.g.* (10), would adopt a chair–twist boat conformation. The related bicyclic compound (12) provided additional evidence on this point. The conformation of (12) was determined by complete analysis of the ^1H n.m.r. spectrum at 400 MHz; it was shown

Table 3. M.p.s and analytical data for compounds (4)—(12)

Compound	M.p. (°C)	Found (%)			Formula	Required (%)		
		C	H	N		C	H	N
(4)	185—187	39.8	3.9	17.0	$C_8H_9N_3O_4S$	39.5	3.7	17.3
(5)	156—158	41.9	4.2	16.1	$C_9H_{11}N_3O_4S$	42.0	4.3	16.3
(6)	119—121	44.3	4.75	15.5	$C_{10}H_{13}N_3O_4S$	44.3	4.8	15.5
(7)	209—211	46.45	5.6	10.65	$C_{10}H_{14}N_2O_4S$	46.5	5.4	10.85
(8)	165—168	48.6	5.9	10.2	$C_{11}H_{16}N_2O_4S$	48.5	5.9	10.3
(9)	170—173	48.35	5.8	10.0	$C_{11}H_{16}N_2O_4S$	48.5	5.9	10.3
(10)	195—196	46.5	5.4	10.6	$C_{10}H_{14}N_2O_4S$	46.5	5.5	10.85
(11)	157—159	46.4	5.5	10.9	$C_{10}H_{14}N_2O_4S$	46.5	5.5	10.85
(12)	212—213	50.75	5.5	13.0	$C_9H_{12}N_2O_4$	50.95	5.7	13.2



Scheme 2. *Reagents:* i, MeOH, HCl; ii, HCHO; iii, PCl_5; iv, AcSK, NaI, acetone

and (10) and their *S*-acetyl methyl esters are in accordance with this conformation. For the *cis*-compound (29) the vicinal couplings for the three ring protons of the twisted boat conformation are *J* 4.1 and 14.2 Hz; also, as expected, there is a narrow multiplet for the C*H*CO_2H proton in the chair conformation. For the *trans*-isomer, as its *S*-acetyl methyl ester (28), the corresponding couplings are 4.97 and 2.2 Hz; this confirms the axial orientation of the CH_2SAc group as shown. A complete analysis of the 400 MHz spectrum was possible, and was confirmed by computer simulation. The coupling constants recorded in Table 1 uniquely defined the conformation of the bicyclic ring system. Subsequently, an *X*-ray diffraction study of the protected compound (28) confirmed this assignment of conformation. Investigation of the deprotected compounds by n.m.r., though limited by overlapping multiplets, confirmed that the rigid conformations of the protected compounds were maintained in the free thiol acids.

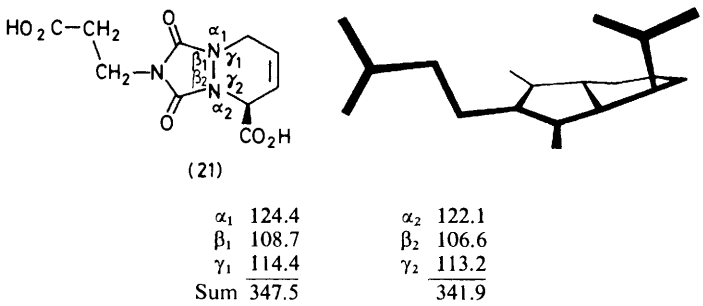


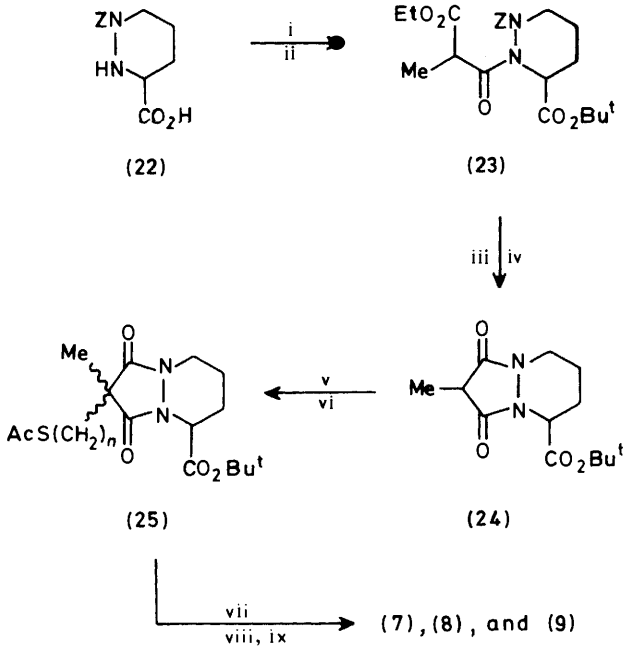
Figure 4. The *X*-ray structure of the triazolopyridazinedicarboxylic acid (20) illustrating the bond angles around the N—N group (*X*-ray data, Daly 1981, unpublished)

to adopt the expected chair–twist boat conformation. The coupling constants, which define this unique conformation, are recorded in Table 1.

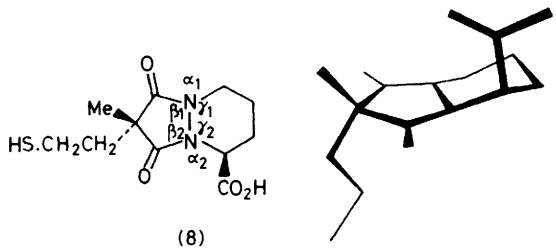
The strong preference of the carboxy group for the axial orientation to relieve ' A ' strain in the bicyclic compound (12) is evidently transmitted through the planar amide bonds to the other ring which is, therefore, held in a fixed conformation with the CH_2CH_2 fragment staggered. This important observation provides a basis for postulating the conformations of the *cis*- and *trans*-diastereoisomers of the bicyclic thiol structure. In each case the ring conformation is identical with that in the unsubstituted analogue. The *cis*- and *trans*-compounds, (11) and (10) respectively, have the CH_2SH function equatorial and axial, respectively (Figure 6). The amide bonds are planar in each case.

The n.m.r. spectra of the *cis*- and *trans*-compounds (11)

Requirements for Binding to the Active Site of Angiotensin Converting Enzyme.—The arrays of the three functions, amide, carboxy group, and methanethiol, in the three bicyclic systems have been compared with the same functions in favoured conformations of captopril using computer graphics. The amide bonds have been matched, the carboxy groups compared, and the conformationally feasible positions of the thiol group have been traced. Figure 7a indicates the matching of captopril with compound (10). Figure 7b is a representation to scale of the mesh trace of the locus common to compound (10) and captopril ($d_{ss}^1 > 0.5$ Å). This process can be carried out for the individual inhibitors (4)—(6) and (8)—(11), producing in each case a restricted thiol locus which corresponds to part of the whole potential locus of captopril. It is postulated that *in vitro* activities, as inhibitors of A.C.E., of conformationally restricted inhibitors are directly dependent



Scheme 3. n = 1 or 2. *Reagents:* i, $Me_2C{=}CH_2,H^+$; ii, EtO_2CCMe, COCl, NaOH, H_2O–CH_2Cl_2; iii, H_2, Pd–C; iv, AcOH, 100 °C; v. NaH, DMF; vi, $AcS(CH_2)_nBr$; vii, separate diastereoisomers; viii, TFA; ix, aq. NH_3



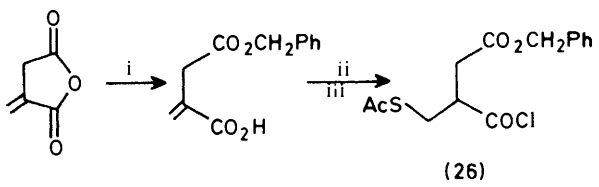
α_1	127.8	α_2	121.4
β_1	111.1	β_2	108.6
γ_1	116.7	γ_2	115.4
Sum	355.6		345.4

Figure 5. The *X*-ray structure of the thiol (9) illustrating the bond angles around the N–N group (*X*-ray data, Daly 1981, unpublished

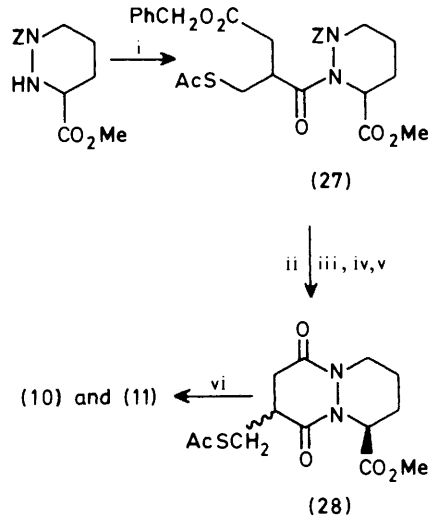
on the ability to achieve the best fit on the enzyme site. The mesh plot (Figure 8) relates the position of the sulphur atom to the biological activity (Γ) of each compound. Consequently, this mesh plot provides some basis for predicting the biological activities of new rigid structures with the appropriate binding functions. It is interesting to observe that one of the two small areas, Γ_6, associated with the highest level of biological activity for the bicyclic compounds corresponds closely to the position for sulphur in captopril bound to penicillopepsin,[8] as observed in *X*-ray diffraction studies.

Assuming standard geometry for the Zn · · · S–CH_2 fragment [19] and the fact that the torsion angle Zn · · · S–CH_2–CHMe is likely, for steric reasons, to arise from a *trans* conformation, the scaled representation in Figure 9 of captopril bound to the catalytic site of A.C.E. can be proposed.

This scaled plot enables the design of new rigid bicyclic structures which are accommodated more closely to the postulated optimum array. We shall report on experiments with this objective in subsequent publications.



Scheme 4. *Reagents:* i, $PhCH_2OH$; ii, AcSH; iii, PCl_5



Scheme 5. *Reagents:* i, (26), aq. NaOH–CH_2Cl_2; ii, separate diastereoisomers; iii, HBr, AcOH; iv, PCl_5, DMF; v, pyridine; vi, aq. NaOH, MeOH

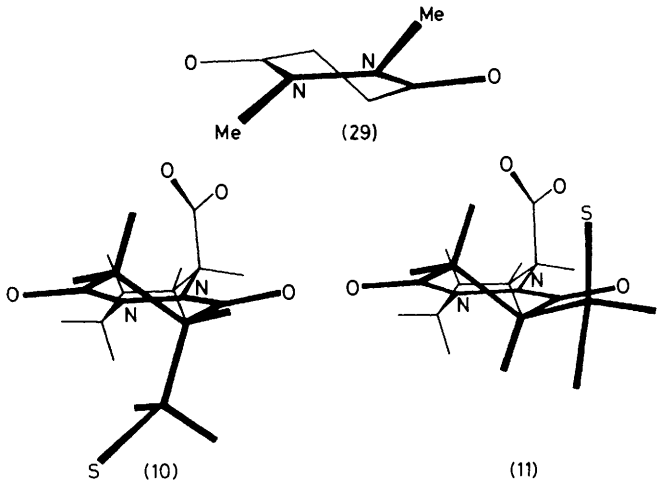


Figure 6. The conformations of compounds (10), (11), and (29)

Experimental

M.p.s were determined on a Buchi melting point apparatus. I.r. spectra were recorded on a Unicam SP 1000 spectrophotometer for Nujol mulls. Mass spectra were recorded on either Varian CH-7 or AEI MS-902 instruments with direct insertion probes. Microanalyses were carried out on a Perkin-Elmer 240 microanalyser by Mr. M. R. Cottrell. Silica gel used for column chromatography was Kieselgel 60, 70—230 mesh (Merck). Ether refers to diethyl ether.

^{1}H *N.M.R. Studies.*—N.m.r. spectra were determined on a Varian XL-100/15 spectrometer, and on Bruker WM-300 and

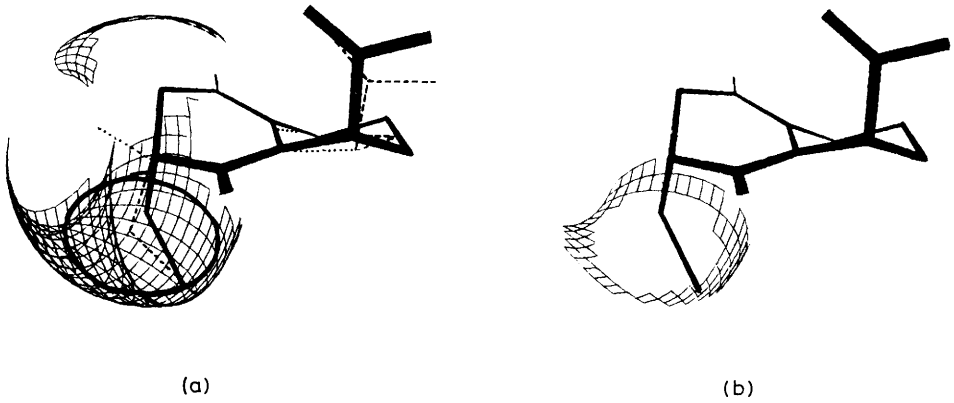


Figure 7. (*a*) Matched molecules of captopril and the bicyclic compound (10); (*b*) the common locus of compound (10) with captopril (S atoms within 0.5 Å of each other)

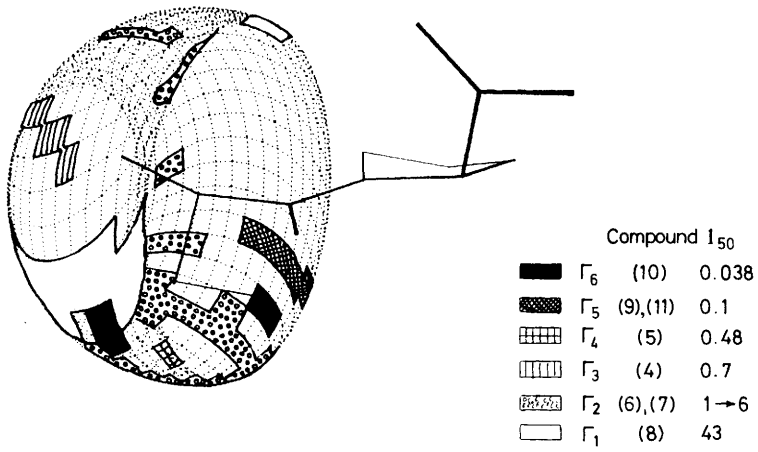


Figure 8. Biological activities assigned to parts of the captopril sulphur atom focus

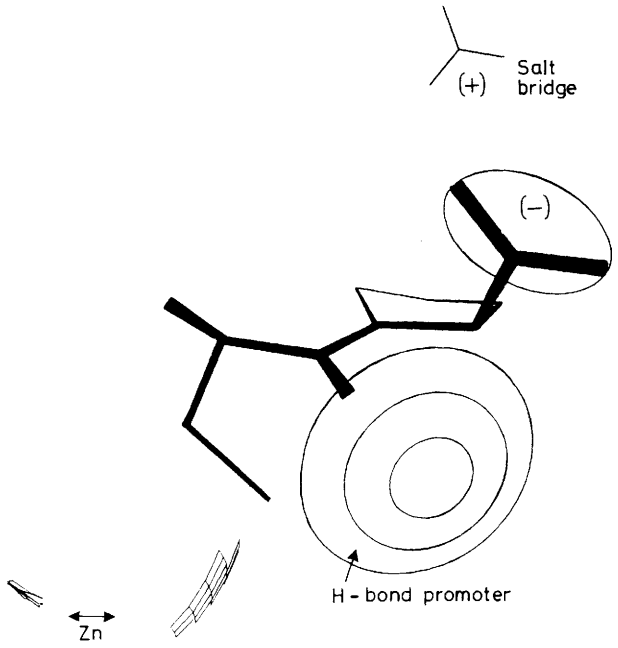


Figure 9. Orientation of captopril for binding to the postulated catalytic site of A.C.E.

WH-400 instruments. We acknowledge the 400 MHz ^1H spectra obtained in the laboratory of Dr. G. Englert, Hoffmann la Roche, Basle, and also the skilful technical assistance in the detailed n.m.r. studies provided by I. W. A. Whitcombe and P. J. Gilbert. Assignments of ^1H n.m.r. spectra were carried out by appropriate decoupling experiments, comparison of the spectra of analogous structures, and also by use of lanthanide shift reagents. After first-order analyses of the spectra yielded approximate chemical shifts and coupling constants, the results were refined by computer simulation of the spectra using SIMEQ II [20] or versions of LAOCOON,[21] providing unambiguous proof of assignments.

Computer Graphics.—Molecular graphics studies were carried out on a DEC PDP 11/40 mini computer having 64K words of memory, floating point arithmetic and memory management, with 5 RKO5 disk drives and the RT-11 operating system. Interfaced to the computer is the Megatek 7000 display terminal with 4096 × 4096 resolution and the accompanying MGS-7000 display processor software. The main program SASA for molecular graphics was written by us in Fortran as a package specifically for drug design: it occupies 120K words of memory partioned into nine segments and two overlay levels. We acknowledge the valuable assistance of Dr. J. E. Pearson on the development of some sections of software.

(*a*) *Input of molecules, display, and plotting.* For known

crystal structures input to the graphics system is by means of the Cartesian co-ordinates which are transformed using a connectivity matrix and displayed either as skeletal or space-filling models. In cases where the crystal structure is not known, molecules are assembled on the screen from a library of parts stored in the computer library or through the *Z* matrix. Typical bond lengths and bond angles for these assembled molecules were obtained from the Cambridge Crystal Data File through CSSR (Daresbury), which is gratefully acknowledged. Manipulations such as rotation or translation are carried out using Fortran routines, continuously updating the set of co-ordinates and projecting them on the display. Alternatively, for faster rotation with larger molecules, the built-in Megatek HCRST unit (Hardware Clip Rotate Scale and Translate) is used successfully. The 3-D effect on the display is obtained by a combination of smooth rotation, depth cueing (intensity variation depending on the distance of the observed part of the molecule from the eye) and/or application of perspective. In plotted structures as shown in this manuscript, tapering of bonds is automatically applied.

(*b*) *Matching of two or more molecules.* Overlaying of two or more molecules is carried out by a combination of linear and quadratic transformations, with a further routine for calculating distance gradients. The linear transformation involving a shift and a rotation tensor is used for matching two points in space where no change in molecular conformation is required. The quadratic transformation allows the matching of two points in space, with the additional possibility of rotation about specified free single bonds to generate the closest match. When the specified points are matched as closely as possible the program prints out the sum of residual cubes between matched points. Selection of matching points is arbitrary; in the applications described here the central amide bond acts as a common fulcrum for all molecules to be matched and hence is used as the main overlayed part of the molecules, following which the thiol groups are matched by the techniques described above.

(*c*) *Locus generation.* The mapping of loci for selected atoms in a molecule is carried out as follows: part of the molecule is held rigid, *e.g.* the acyl proline moiety in captopril, and by allowing free rotation about the remaining free single bonds the movements of the terminal atom (sulphur in the case of captopril) can be mapped, recording a point in space for every (say) 10° of bond rotation, and joining up the resultant dots to form a circle, net or volume, depending on whether one, two, or three degrees of freedom respectively for bond rotation are employed. The matching program can then be used for overlaying the loci generated for different molecules, and hence producing the common locus between the matched pair.

Molecular Mechanics Calculations.—In early attempts to assess conformational stabilities, Van-der-Waals overlap was used as a criterion of steric interactions. Since intermolecular dispersion forces are better approximated by Lennard-Jones potentials, the program used for these purposes incorporates Morse potentials which require fewer computer operations and are therefore faster.[9]

Three parameters define the Morse potential: ε_{min}, the minimum energy at a distance r_m between atoms, and b, the ' steepness ' or softness of the interaction. These calculations were found to be adequate for the operations described in this paper taking into account the limitations of the computer. We are grateful to Dr. R. J. Abraham (Liverpool University) for supplying this program.

X-*Ray Diffraction.*—X-Ray diffraction studies were carried out in the Hoffmann-La Roche Research laboratories, Basle. We are very grateful to Dr. J. J. Daly and Mr. P. Schonholzer for these results which will be reported fully elsewhere. The structures were obtained by direct methods on a Hilger and Watts four circle diffractometer model Y290/PDP8. Co-ordinates of the compounds (21), (8), and (28) are available from Dr. J. J. Daly, Central Research Laboratories, Hoffmann-La Roche, Basle, Switzerland, on request.

2,3,5,8-*Tetrahydro*-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]-*pyridazine*-5-*carboxylic Acid* (4), (5), *and* (6).—(*a*) *Preparation of* 1,2,4-*triazolidine*-3,5-*diones* (14). 4-(2-Chloroethyl)-1-ethoxycarbonylsemicarbazide (23.89 g, 0.114 mmol) (13; $n = 2$) was stirred with aqueous potassium hydroxide (4M; 57 ml, 0.228 mmol) at room temperature for 2 h. The resulting solution was diluted with water and chromatographed on Dowex 50-X-8 (H^+) to give 4-(2-*chloroethyl*)-1,2,4-*triazolidine*-3,5-*dione* (14; $n = 2$) (7.97 g, 42%), m.p. 192—195 °C (from water) (Found: C, 29.2; H, 3.7; N, 25.6. $C_4H_6ClN_3O_2$ requires C, 29.4; H, 3.7; N, 25.7%).

4-(3-*Chloropropyl*)-1,2,4-*triazolidine*-3,5-*dione* (14; $n = 3$), m.p. 168—170 °C (from water) was similarly prepared from the appropriate semicarbazide.

(*b*) *Diels–Alder reactions of* 1,2,4-*triazole*-3,5-*diones. General procedure.* The 4-substituted 1,2,4-triazolidine-3,5-dione (11 mmol) was suspended in dry dioxane (40 ml), and stirred under nitrogen at room temperature. A solution of t-butyl hypochlorite (2.0 ml, 16.7 mmol) in dioxane (10 ml) was added dropwise during 10 min. The resulting red solution was stirred for 45 min, filtered, and evaporated to a red oil.

Without further purification, the red oil was dissolved in dioxane (40 ml) and added dropwise to a stirred solution of penta-2,4-dienoic acid (1.32 g, 13.5 mmol) at room temperature. The red colour of the dienophile was allowed to fade between additions. The resulting yellow solution was evaporated to yield the crude product.

The Diels–Alder adducts were either purified by recrystallisation, or directly esterified, and the resulting esters recrystallised.

The following compounds were prepared by this method. 2-(2-*Chloroethyl*)-2,3,5,8-*tetrahydro*-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]*pyridazine*-5-*carboxylic acid* (16; $n = 2$), m.p. 150—152 °C (from acetonitrile–ether) (Found: C, 41.85; H, 3.8; N, 16.2. $C_9H_{10}ClN_3O_4$ requires C, 41.6; H, 3.85; N, 16.2%).

t-Butyl ester of compound (16; $n = 2$), m.p. 87—88 °C (from hexane) (Found: C, 49.3; H, 5.8; N, 13.15. $C_{13}H_{18}ClN_3O_4$ requires C, 49.4; H, 5.7; N, 13.3%).

Methyl 2-(3-*chloropropyl*)-2,3,5,8-*tetrahydro*-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]*pyridazine*-5-*carboxylate* (16; $n = 3$), m.p. 99—101 °C (from dichloromethane–hexane) (Found: C, 46.2; H, 4.9; N, 14.5. $C_{11}H_{14}ClN_3O_4$ requires C, 45.9; H, 4.9; N, 14.6%).

(*c*) *Modification of the* 2-*substituent.* 2,3,5,8-*Tetrahydro*-2-*mercaptomethyl*-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]*pyridazine*-5-*carboxylic acid* (4). 2,3,5,8-Tetrahydro-1,3-dioxo-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine-5-carboxylic acid [12] (18) was refluxed in methanolic hydrogen chloride for 2 h. Evaporation gave the corresponding methyl ester, m.p. 210—213 °C. The above methyl ester was heated with excess of 40% aqueous formaldehyde for 4 h. Evaporation gave the hydroxymethyl compound (19), m.p. 121—125 °C, which was used without further purification.

The above hydroxymethyl compound (19) (3.97 g, 16.5 mmol) was suspended in dry ether (300 ml) and cooled to 0 °C. Phosphorus pentachloride (3.49 g, 16.8 mmol) was added in four portions, and the mixture stirred at room temperature for 22 h. Evaporation gave a solid residue which was purified by chromatography on silica gel to give *methyl*-2-

chloromethyl-2,3,5,8-*tetrahydro*-1,3-*dioxo*-1H-[1,2,4]*triazolo*-[1,2-a]*pyridazine*-5-*carboxylate* (3.24 g, 76%), m.p. 164—165 °C. The chloromethyl compound (3.24 g, 12.5 mmol), potassium thioacetate (2.29 g, 20 mmol), and a catalytic amount of sodium iodide were refluxed in acetone with vigorous stirring for 19 h. The solvent was evaporated, and the residue partitioned between ethyl acetate (250 ml) and brine (200 ml). The organic layer was separated, dried, and evaporated to a yellow oil, which was chromatographed to give the *acetylthio derivative* (20) (2.19 g, 59%), m.p. 110—111 °C (Found: C, 44.1; H, 4.4; N, 13.9. $C_{11}H_{13}N_3O_5S$ requires C, 44.1; H, 4.4; N, 14.0%).

Hydrolysis of the above compound (20) (440 mg, 1.5 mmol) in methanolic sodium hydroxide (1M; 6 ml) at room temperature gave, on acid work-up, the *title compound* (4) (150 mg, 42%), data in Table 3.

2,3,5,8-*Tetrahydro*-2-(2-*mercaptoethyl*)-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]*pyridazine*-5-*carboxylic acid* (5). Treatment of the t-butyl ester of the corresponding chloro compound with potassium thioacetate exactly as described above gave the fully protected thiol, m.p. 102—103 °C. Cleavage of the t-butyl ester in trifluoroacetic acid, followed by hydrolysis in aqueous ammonia gave the *title compound*, data in Table 3.

2,3,5,8-*Tetrahydro*-2-(3-*mercaptopropyl*)-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]*pyridazine*-5-*carboxylic acid* (6). Treatment of the methyl ester of the corresponding chloro compound with potassium thioacetate exactly as described above, followed by hydrolysis in aqueous methanolic sodium hydroxide, gave the *title compound*, data in Table 3.

3-{5-*Carboxy*-2,3,5,8-*tetrahydro*-1,3-*dioxo*-1H-[1,2,4]*triazolo*[1,2-a]*pyridazin*-2-*yl*}*propionic acid* (21). In a similar manner *via* the appropriate 4-substituted 1,2,4-triazolidine-3,5-dione, the title compound was prepared: m.p. 172—175 °C (from acetonitrile) (Found: C, 44.8; H, 4.2; N, 15.8. $C_{10}H_{11}N_3O_6$ requires C, 44.65; H, 4.12; N, 15.62%); n.m.r. details in Table 1.

Hexahydro-1,3-*dioxo*-1H-*pyrazolo*[1,2-a]*pyridazine*-5-*carboxylic Acids* (7), (8), *and* (9).—(*a*) *t-Butyl hexahydro*-2-*methyl*-1,3-*dioxo*-1H-*pyrazolo*[1,2-a]*pyridazine*-5-*carboxylate* (24). t-Butyl 1-benzyloxycarbonylhexahydropyridazine-3-carboxylate (7.78 g, 0.024 mmol) was dissolved in dichloromethane (65 ml) and treated simultaneously with ethyl methylmalonyl chloride (4.0 g, 0.024 mmol) in dichloromethane (30 ml) and aqueous sodium hydroxide (1M; 24 ml, 0.024 mmol) with vigorous stirring. The organic layer was separated, washed with water, dried and evaporated to leave a brown oil which was chromatographed to give compound (23) (3.92 g, 36%).

The above compound (3.90 g, 8.7 mmol) was dissolved in methanol (100 ml) and hydrogenated over 10% palladium-on-charcoal (0.39 g) at atmospheric pressure. The solution was filtered free of catalyst and evaporated to leave a colourless solid. The solid was dissolved in acetic acid (150 ml) and heated at 100 °C for 2 h. Evaporation left a yellow solid which was purified by chromatography to give the title compound as a colourless solid (1.39 g, 60%), m.p. 127—128 °C (from chloroform–hexane) (Found: C, 58.1; H, 7.5; N, 10.5. $C_{13}H_{20}N_2O_4$ requires C, 58.2; H, 7.5; N, 10.4%).

(*b*) *Alkylation of the ester* (24) *with* S-*bromomethyl thioacetate*. Sodium hydride (80%; 0.8 g, 0.0267 mmol) was suspended in dry dimethylformamide (DMF) (50 ml). A solution of (24) (7.08 g, 0.0264 mmol) in DMF (60 ml) was added, and the mixture stirred until gas evolution was complete. *S*-Bromomethyl thioacetate (4.98 g, 0.0295 mmol) was added, and the mixture stirred under nitrogen at 75 °C for 5 h. The mixture was evaporated to a yellow solid, which was then dissolved in water. The solution was acidified and extracted with chloroform (2 × 200 ml). The extracts were dried and evaporated to a yellow oil. The mixture of alkylated products (25; $n = 1$) was separated into two diastereoisomers by chromatography on silica gel.

(*c*) *Alkylation of the ester* (24) *with* S-2-*bromoethyl thioacetate*. Compound (24) was alkylated with *S*-2-bromoethyl thioacetate using sodium hydride as base exactly as described above, to give a diastereoisomeric mixture of alkylated products (25; $n = 2$) which could be separated by chromatography.

(*d*) *Preparation of the free thiol carboxylic acids* (7)—(9). *General procedure*. The *S*-acetyl t-butyl ester (single diastereoisomer) (*ca.* 2 mmol) was dissolved in trifluoroacetic acid (TFA) (10 ml) and allowed to stand at room temperature for 1 h. Evaporation of the TFA left a brownish oil which was dissolved in a 1 : 1 mixture of water and ammonia (*d* 0.88) (*ca.* 50 ml) and stirred under nitrogen for 2 h. The mixture was acidified to pH 1 with concentrated hydrochloric acid (cooling necessary), saturated with sodium chloride, and extracted with chloroform (3 × 50 ml). The extracts were dried, and evaporated to give the crude thiol acids (7), (8), and (9), which were purified by recrystallisation or chromatography. Data for compounds (7), (8), and (9) are summarised in Table 3.

Octahydro-6,9-*dioxopyridazo*[1,2-a]*pyridazines* (10), (11), *and* (12).—(*a*) 2-*Acetylthiomethyl*-3-*benzyloxycarbonylpropionyl chloride* (26). Itaconic (methylenesuccinic) anhydride (56 g, 0.5 mmol) and benzyl alcohol (54 g, 0.5 mmol) were heated together on a steam bath for 2 h to give monobenzyl itaconate, m.p. 77—82 °C, used without further purification.

Monobenzyl itaconate (20 g, 0.091 mol) and thioacetic acid (9.5 ml) were heated on a steam bath for 3 h. Excess of thioacetic acid was removed under vacuum and the residue was dissolved in ether (80 ml) and stirred with phosphorus pentachloride (19.0 g, 0.091 mol). The volatile materials were removed to give the acid chloride (26) (27 g, 94%) as a yellow oil, used without further purification.

(*b*) 1-*Benzyl* 3-*methyl* 2-(2-*acetylthiomethyl*-3-*benzyloxycarbonylpropionyl*)*hexahydropyridazine*-1,3-*dicarboxylate* (27). Methyl 1-benzyloxycarbonylhexahydropiperazine-3-carboxylate (2.50 g, 9 mmol) was dissolved in dichloromethane (40 ml). The solution was stirred rapidly whilst a solution of (26) (3.22 g, 10.2 mmol) in dichloromethane (15 ml) and aqueous sodium hydroxide (0.5M; 20 ml, 10 mmol) were added simultaneously during 10 min. The mixture was stirred for a further 3.5 h. The organic layer was separated, washed with aqueous sodium hydroxide and water, dried, and evaporated to a yellow oil (5.15 g). The crude product was separated by chromatography to give two diastereoisomers, isomers of the title compound as colourless oils.

Octahydro-8-*mercaptomethyl*-6,9-*dioxopyridazo*[1,2-a]-*pyridazine*-1-*carboxylic Acid* (10) *and* (11).—The less polar diastereoisomer of (27) (8.76 g, 0.016 mol) was dissolved in acetic acid (20 ml). A solution of hydrogen bromide in acetic acid (45%, 60 ml) was added, and the mixture allowed to stand at room temperature for 1.5 h. The mixture was evaporated to a yellow oil which was dissolved in DMF (100 ml), cooled to 0 °C, and treated with phosphorus pentachloride (3.60 g, 0.017 mmol). After the mixture had been stirred at 0 °C for 0.5 h, and at room temperature for 2 h, pyridine (16 ml) was added, and the mixture stirred for a further 2 h, then evaporated and the residue dissolved in ethyl acetate. The solution was washed with dilute hydrochloric acid (70 ml), saturated sodium hydrogen carbonate (150 ml), and brine (100 ml), dried and evaporated. The major product was isolated by chromatography, and recrystallisation from ethyl acetate–

hexane gave *methyl 8-acetylthiomethyloctahydro-6,9-dioxopyridazo*[1,2-a]*pyridazine-1-carboxylate* (28) (0.54 g, 11%), m.p. 84—86 °C (Found: C, 49.85; H, 5.9; N, 8.9. $C_{13}H_{18}N_2O_5S$ requires C, 49.7; H, 5.8; N, 8.9%). N.m.r. spectroscopy and *X*-ray diffraction indicated that this diastereoisomer had the *SS*/*RR* configuration.

The above compound (240 mg, 0.76 mmol) was hydrolysed in methanol (1.5 ml) and aqueous sodium hydroxide (1M; 3 ml, 3 mmol) at room temperature under nitrogen. Acid work-up gave the *title compound* (10) (80 mg, 41%), data given in Table 3.

The other diastereoisomer (*RS*/*SR* racemate) was obtained as a minor product of the above route in an impure form. By an independent route a pure sample of this diastereoisomer (11) was obtained. We are grateful to Dr. G. Lawton for the preparation and purification of this diastereoisomer, whose stereochemistry was determined by n.m.r. spectroscopy on the protected *S*-acetyl methyl ester (29). The deprotected *RS*/*SR* isomer (11) had m.p. 157—159 °C (Found: C, 46.4; H, 5.5; N, 10.9. $C_{10}H_{14}N_2O_4S$ requires C, 46.5; H, 5.5; N, 10.85%).

1,4,6,9-*Tetrahydro*-6,9-*dioxopyridazo*[1,2-a]*pyridazine*-1-*carboxylic Acid* (12).—*Methyl* 1,4,6,9-*tetrahydro*-6,9-*dioxopyridazo*[1,2-a]*pyridazine*-1-*carboxylate*. Maleic hydrazide (1.12 g, 10 mmol) was suspended in dichloromethane (50 ml). Methyl penta-2,4-dienoate (1.12 g, 10 mmol) was added and the mixture stirred at room temperature. Lead tetra-acetate (4.44 g, 10 mmol) was added in portions during 1 h. After the addition, the mixture was stirred overnight at room temperature then filtered. Following evaporation, the residual brown oil was chromatographed on silica gel, a yellow sticky solid (0.27 g) being eluted with chloroform. Recrystallisation from dichloromethane–hexane gave the title compound as yellow crystals (0.16 g, 7%), m.p. 140—142 °C (Found: C, 53.9; H, 4.5; N, 12.5. $C_{10}H_{10}N_2O_4$ requires C, 54.05; H, 4.50; N, 12.61%); δ($CDCl_3$) 3.80 (3 H, s), 4.50 (2 H, m), 5.72 (1 H, m), 6.16 (2 H, m), and 7.00 (2 H, d).

Octahydro-6,9-*dioxopyridazo*[1,2-a]*pyridazine*-1-*carboxylic acid* (12). A solution of methyl 1,4,6,9-tetrahydro-6,9-dioxopyridazo[1,2-*a*]pyridazine-1-carboxylate (1.0 g, 4.4 mmol) in methanol (25 ml) was hydrogenated over platinum oxide (100 mg) during 20 h. Filtration and evaporation of the filtrate gave *methyl octahydro*-6,9-*dioxopyridazo*[1,2-a]-*pyridazine*-1-*carboxylate* (1.0 g, 98%). Crystallisation from ethyl acetate gave an analytical sample, m.p. 104 °C (Found: C, 53.0; H, 6.05; N, 12.6. $C_{10}H_{14}N_2O_4$ requires C, 53.1; H, 6.25; N, 12.4%); δ($CDCl_3$) 1.3—3.0 (9 H, m), 3.75 (3 H, s, OMe), 4.7 (1 H, m, NCH), and 5.35 (1 H, m, NCH); *m*/*z* 226 (M^+, 30%), 167 (100), and 139 (25); $\nu_{max.}$ 1 750, 1 700, and 1 680 cm^{-1}.

Methyl octahydro-6,9-dioxopyridazo[1,2-*a*]pyridazine-1-carboxylate (0.5 g, 2.2 mmol) was stirred in methanol (5 ml) and treated with NaOH (0.13 g, 3.2 mmol) in water (5 ml). The solution was stirred at 20 °C for 1.5 h and Zerolit 225 ion exchange resin added. The resin was filtered and washed with water. After evaporation, the residue was recrystallised from dioxane–hexane to give *octahydro*-6,9-*dioxopyridazo*[1,2-a]-*pyridazine*-1-*carboxylic acid* (12) (0.3 g, 64%), m.p. 212—213 °C (Found: C, 50.75; H, 5.5; N, 13.0. $C_9H_{12}N_2O_4$ requires C, 50.95; H, 5.7; N, 13.2%); n.m.r. data in Table 3; *m*/*z* 212 (M^+, 40%), 167 (100), and 139 (40).

References

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Received 25*th May* 1983; *Paper* 3/845