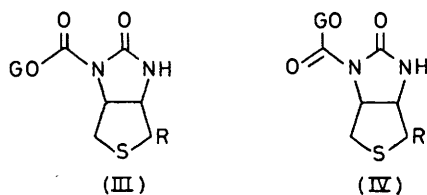
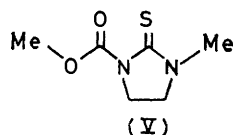


Considerable controversy exists concerning the precise mechanism of reactions (1) and (2), and the nature of the enzyme-biotin-CO₂ complex.¹ Recent experiments² have suggested that *N*-carboxybiotin (II) or a derivatized form is the reactive species. Further suggestions³ are that an *N*-carboxybiotin-acceptor enzyme complex exists prior to CO₂ transfer.

Previous crystallographic studies of biotin and biotin analogues have emphasized the stereochemistry of the bicyclic ring system,^{4,5} the close proximity of the valeryl side-chain to the ureido-ring,^{4,5} the role of the sulphur atom and the tetrahydrothiophen ring,^{5,6} and the potential significance of hydrogen bonding on the ease of polarization of the ureido-bond.⁵ Little attention, however, has been given to the conformation of the carboxy-group. If (II) is bound to a group G (*e.g.*, an enzyme, a metal ion, or a proton) as has been previously suggested,^{3,7} two possible conformations exist which preserve the expected co-planarity of the carboxy-ureido structural unit, the U-shaped conformer (III), and the sickle-shaped con-



former (IV). If G = H, (IV) would be preferred owing to hydrogen bonding to the ureido carbonyl,* but in the absence of hydrogen bonding no *a priori* conformation suggests itself. It was therefore of interest to us to carry out a single-crystal X-ray structure determination of the title compound (V),⁸ a potential model for an enzyme-carboxybiotin intermediate.



EXPERIMENTAL

Compound (V) was prepared by the method previously reported.⁸ Crystals were obtained by slow evaporation of a carbon tetrachloride solution. A single crystal *ca.* 0.38 × 0.42 × 0.29 mm³ with well developed forms {100}, {010},

* In 1-*N*-carboxybiotin bis-*p*-bromoanilide⁴ the sickle-conformation is stabilized by a hydrogen bond between the ureido carbonyl and the anilide.

² R. B. Guchhait, S. E. Polakis, P. Dimroth, E. Stoll, J. Moss, and M. D. Lane, *J. Biol. Chem.*, 1974, **249**, 6633; R. B. Guchhait, S. E. Polakis, D. Hollis, C. Fenselaw, and M. D. Lane, *ibid.*, p. 6646; S. E. Polakis, R. B. Guchhait, E. E. Zwergel, and M. D. Lane, *ibid.*, p. 6657.

³ (a) C. H. Fung, R. K. Gupta, and A. S. Mildvan, *Biochem.*, 1975, **15**, 85; (b) A. S. Mildvan and M. C. Scrutton, *ibid.*, 1967, **6**, 2978; (c) J. Retej and F. Lynen, *Biochem. Z.*, 1965, **342**, 256; (d) D. B. Northrup and H. G. Wood, *J. Biol. Chem.*, 1969, **244**, 5801.

⁴ C. Bonnemere, J. A. Hamilton, L. K. Steinrauf, and J. Klappe, *Biochem.*, 1965, **4**, 240.

{001}, and {101} was mounted on a glass fibre in arbitrary orientation with a minimum amount of epoxy-adhesive. All diffraction measurements were carried out on an Enraf-Nonius CAD 4 Kappa computer-controlled diffractometer, with pulse-height analyser, by use of graphite-monochromated Mo-*K*_α radiation at a take-off angle of 3.5°.

A preliminary survey of the positions and intensities of selected reflections established that the crystal system is monoclinic, and subsequent least-squares refinement of 15 carefully centred reflections in the range 28 to 32° (2θ) yielded precise lattice constants. The intensity at each reciprocal lattice point in the two octants *hkl* and *h̄k̄l*, 8° < 2θ < 50° (0.098 < sin θ/λ < 0.595) was measured by the ω 2θ scan technique. The 2θ scan range was computed as (2.2 + 0.8 tan θ)°, and this range was then extended 25% on each end to produce background counts. Throughout data collection, two reflections were measured periodically to monitor electronic and crystal instabilities, neither of which was detected. In addition, a third reflection was monitored for angular displacement. No movement >0.1° in any angle was detected.

A total of 1 571 unique reflection intensities was measured, of which 129 were systematically absent (*h*0*l*, *h* = 2*n* + 1; 0*k*0, *k* = 2*n* + 1), and 575 were considered unobserved [*I* < 3σ(*I*)]. Lorentz and polarization (Lp) corrections were applied to the integrated intensities to yield structure amplitudes.

Crystal Data.—C₆H₁₀N₂O₂S, *M* = 174. Monoclinic, *a* = 15.225 (2), *b* = 7.110 (1), *c* = 7.677 (1) Å, β = 93.80 (8)°, *U* = 829 Å³, *D*_m(floatation) = 1.40 (5), *Z* = 4, *D*_c = 1.39 g cm⁻³. Mo-*K*_α radiation λ = 0.7107 Å; μ(Mo-*K*_α) = 3.4 cm⁻¹, estimated transmission range 0.81 to 0.90. Space group *P*2₁/*a*.

Structure Solution and Refinement.—Routine application of the multiple-solution phasing procedure MULTAN⁹ generated eight phase sets. The three phase sets having the highest and identical figures-of-merit (absolute and combined of 1.1165 and 2.0000 respectively) differed only in choice of origin. An *E* map clearly showed the positions of all non-hydrogen atoms, and a structure-factor calculation based on those positions yielded *R* 23.7%. Four cycles of full-matrix least squares with unit weights and isotropic thermal parameters reduced *R* to 16.7%. A difference-Fourier map revealed the positions of all ten hydrogen atoms, and several cycles of weighted full-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms reduced *R* to 4.7% and weighted *R'* to 6.5%.

It was observed that the six strongest reflections suffered extinction, and these were excluded from the final cycles. Also, 302 of the unobserved reflections were included in the final cycles since for these |*F*_o| ≥ |*F*_c| > 0. The final *R* values for 861 observed reflections were 3.6% and *R'* 4.2%, and for the 575 unobserved reflections *R* 33.3%. The error-of-fit with 1 163 contributing reflections and 140 variables was 1.21. All last-cycle shifts in the non-hydrogen atomic

⁵ G. T. DeTitta, J. W. Edmonds, W. Stallings, and J. Donohue, *J. Amer. Chem. Soc.*, 1976, **98**, 1920.

⁶ C. S. Chem, R. Parthasarathy, and G. T. DeTitta, *J. Amer. Chem. Soc.*, 1976, **98**, 4983.

⁷ H. Kohn, *J. Amer. Chem. Soc.*, 1976, **98**, 3690; E. Ryder, C. Gregolin, H. C. Chang, and M. D. Lane, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 1455; A. F. Hegarty, R. F. Pratt, T. Giudici, and T. C. Bruice, *J. Amer. Chem. Soc.*, 1971, **93**, 1428.

⁸ H. Kohn, M. J. Cravey, J. H. Arceneaux, R. L. Cravey, and M. R. Wilcott, jun., *J. Org. Chem.*, 1977, **42**, 941.

⁹ G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

positional parameters were $<0.5\sigma$, and only one of the 140 parameters changed by $>1.0\sigma$ (1.48σ for z of one methyl hydrogen). A final ΔF map showed the greatest residual electron density (*ca.* $0.5 \text{ e}\text{\AA}^{-3}$) about the methyl groups, presumably due to nearly free rotation in the solid state at 22°C .

The atomic scattering factors for non-hydrogen atoms were taken from ref. 10, while those for hydrogen were taken from ref. 11. Computer programs used in this work, in addition to the Enraf-Nonius/DEC PDP8 diffractometer control programs,¹² were REDUCE,¹³ SORTMERGE,¹⁴ CLASSIFY,¹⁴ WEIGHT,¹⁴ MULTAN,⁹ GENLS,¹⁵ 'X-ray '72'¹⁶ and ORTEP II.¹⁷

RESULTS AND DISCUSSION

Final atomic parameters are listed in Table 1, and molecular dimensions in Table 2. Details of least-

TABLE 1

Atomic positional parameters ($\times 10^4$, for H atoms $\times 10^3$), with estimated standard deviations in parentheses. Hydrogen atoms are numbered according to the carbon atom to which they are bonded

Atom	x	y	z
C(1)	1 711(3)	2 173(7)	4 229(7)
C(2)	585(2)	4 429(4)	3 013(3)
C(3)	-527(2)	2 287(4)	1 923(5)
C(4)	284(2)	1 214(5)	2 557(7)
C(5)	-816(2)	5 755(4)	1 674(4)
C(6)	-2 262(3)	6 367(7)	485(7)
N(1)	874(1)	2 672(3)	3 283(3)
N(2)	-255(1)	4 283(3)	2 129(3)
O(1)	-659(1)	7 386(3)	1 724(3)
O(2)	1 606(1)	5 012(3)	1 129(3)
S	1 117(1)	6 369(1)	3 625(1)
H(11)	216(3)	174(6)	335(6)
H(12)	165(3)	127(6)	508(6)
H(13)	188(3)	319(7)	487(6)
H(31)	-103(2)	207(4)	273(4)
H(32)	-68(2)	210(4)	77(4)
H(41)	22(3)	17(6)	342(5)
H(42)	60(3)	67(6)	152(6)
H(61)	-232(3)	725(6)	115(5)
H(62)	207(3)	678(7)	-70(7)
H(63)	-277(3)	555(6)	9(6)

squares planes, thermal parameters, and torsion angles, final observed and calculated structure factors are listed in Supplementary Publication No. SUP 22092 (13 pp., 1 microfiche).^{*} Figure 1 shows the structure, and Figure 2 is a stereodiagram of the unit cell packing.

The molecule is approximately planar with the U-conformation. Preference for the U- over the sickle-conformation is probably not due to intramolecular steric interactions, and we suspect only definitive calculations will be able to distinguish the two on the basis of total molecular energy. Molecular planarity is expected on the basis of the large number of possible resonance

^{*} See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1977, Index issue.

¹⁰ D. Cromer and J. Mann, *Acta Cryst.*, 1968, **A24**, 321.

¹¹ R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

¹² Enraf-Nonius Corporation, Delft, Holland, 1974.

¹³ J. Korp, University of Houston, 1976.

structures and the implied conjugation in the seven-atom carboxythioureido π -system.

TABLE 2

Bond distances (\AA) and angles ($^\circ$)

(a) Distances			
C(1)-N(1)	1.467(5)	N(2)-C(5)	1.381(4)
N(1)-C(2)	1.336(4)	C(3)-C(4)	1.504(5)
N(1)-C(4)	1.459(4)	C(5)-O(1)	1.184(4)
C(2)-S	1.652(3)	C(5)-O(2)	1.355(3)
C(2)-N(2)	1.412(3)	O(2)-C(6)	1.451(5)
N(2)-C(3)	1.484(4)		
(b) Angles			
C(1)-N(1)-C(2)	124.7(3)	C(3)-N(2)-C(5)	122.3(2)
C(2)-N(1)-C(4)	114.7(2)	N(2)-C(3)-C(4)	103.5(2)
C(1)-N(1)-C(4)	120.6(3)	N(1)-C(4)-C(3)	103.6(2)
N(1)-C(2)-N(2)	106.5(2)	N(2)-C(5)-O(1)	127.9(2)
N(1)-C(2)-S	125.9(2)	N(2)-C(5)-O(2)	107.7(2)
N(2)-C(2)-S	127.6(2)	O(1)-C(5)-O(2)	124.4(2)
C(2)-N(2)-C(3)	111.1(2)	C(5)-O(2)-C(6)	115.1(2)
C(2)-N(2)-C(5)	126.2(2)		
H(11)-C(1)	1.04(6)	H(41)-C(4)	1.01(6)
H(12)-C(1)	0.92(6)	H(42)-C(4)	1.04(7)
H(13)-C(1)	0.90(6)	H(61)-C(6)	0.81(7)
H(31)-C(3)	1.04(4)	H(62)-C(6)	1.01(8)
H(32)-C(3)	0.91(4)	H(63)-C(6)	1.00(6)

Surprisingly, significant deviations from planarity are observed; the root-mean-square deviation from the mean molecular plane (excluding hydrogen atoms) is 0.082 \AA , and individual deviations range from -0.12 to 0.12 \AA . The three primary sites of non-planarity are N(2), and

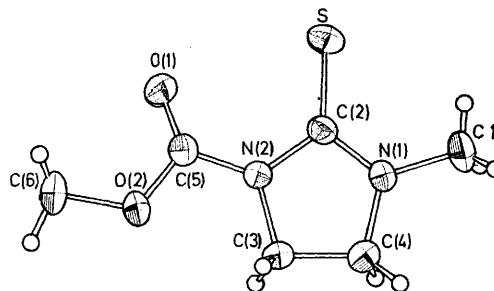


FIGURE 1 View of the molecule of (V), showing the numbering scheme used in the crystal structure analysis

the C(5)-N(2) and C(3)-C(4) bonds. Of the four three-valent moieties in the carboxythioureido π -system, only the three bonds about N(2) deviate significantly from coplanarity. This 'pyramiding' of N(2) may be assessed by the half-open cone angle¹⁸ at N(2) of 87.93° , 2.07° from the 90° expected for a planar moiety [for N(1), C(2), and C(5), deviations from 90° are 0.08, 0.15, and 0.07°]. The result is a depression of the C(5)-N(2) bond by 3.0° below the thioureido plane [N(1)-C(2)-N(2)] with con-

¹⁴ S. F. Watkins, University of Houston, 1976.

¹⁵ A. C. Larson, Los Alamos National Laboratory, 1973.

¹⁶ J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, Technical Report TR 192, Computer Science Center, University of Maryland, 1972.

¹⁷ C. K. Johnson, Technical Report ORNL 5138, Oak Ridge National Laboratory, Tennessee, 1976.

¹⁸ J. M. Whitfield, S. F. Watkins, G. B. Tupper, and W. H. Baddley, *J.C.S. Dalton*, 1977, 407.

comitant loss of overlap between these two parts of the π system.

Further loss of overlap results from an 8.4° torsion about the C(5)-N(2) bond. Thus, the total dihedral angle of 8.9° between the carboxy- and the thioureido-planes represents a partial decoupling of the carboxy- and thioureido- π -systems. No significantly short inter- or

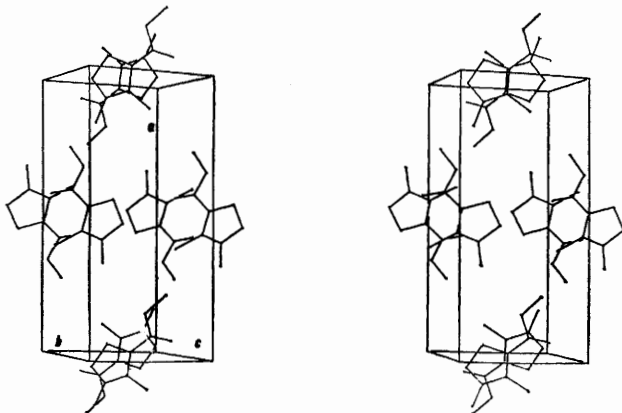


FIGURE 2 Stereodiagram of the crystal structure of (V)

intra-molecular contact distances are evident, so the observed non-planarity must be due to inherent electronic effects.

The five-membered ring is not planar and is best described as a twisted C_2 half-chair with the ethylene carbon atoms above and below the thioureido-plane. The asymmetry parameters are ΔC_2 0.81° (Duax and Norton¹⁹) and $\Delta = 7.07^\circ$, $\phi_m = 8.0^\circ$ (Altona *et al.*²⁰). The nearly C_2 conformation is caused by an 8° torsion about the ethylene bond. Ethylene torsions were also observed in dethiobiotin⁶ (22.2°) and α -phenyltetramethylnitronitroxide²¹ (20° averaged over two independent molecules). In the latter compound torsion about the ethylene bond staggers the methyl groups to reduce steric interaction, and it seems reasonable to suppose that in dethiobiotin, steric interaction in the *cis*-methyl-methylene system also accounts for this torsion. In (V), the hydrogen atoms require less torsion to in-

crease their internuclear separations [$H(31) \cdots H(41)$ and $H(32) \cdots H(42)$, mean $2.31(7)$ Å].

In addition to the angular distortions, bond-length dissimilarities add further to the asymmetry of the ring. There have been numerous structural studies of thiourea, either as a pure crystalline substance^{22,23} or as a non-bonded adduct in ionic host lattices,²⁴⁻²⁸ and the observed dimensions are consistent with mean distances C-S $1.697(2)$ and C-N $1.335(2)$ Å. In (V), C(2)-S is $1.652(3)$ Å, significantly shorter than in thiourea, and is among the shortest distances listed for heterocyclic compounds in ref. 29. The C(2)-N(1) distance [$1.336(4)$ Å] represents a partial double bond, identical to that in thiourea, but C(2)-N(2) is significantly longer [$1.412(3)$ Å]. The C(5)-N(2) bond length of $1.381(4)$ Å is between the two ring C-N values, implying that the bond orders for C(2)-N(2), C(5)-N(2), and C(2)-N(1) increase in that order. The three remaining C-N bonds do not significantly deviate from their mean value of $1.470(3)$ Å, the expected (three-valent N) single-bond distance,²⁹ and all of the other C-O, C-C, and C-H bond lengths are as expected.²⁹

The bent and twisted orientation of the carboxy-group relative to the thioureido-plane and the dissimilarities between the three C-N bonds of the π system are consistent with incomplete conjugation between the carboxy- and thioureido-moieties. This observation strengthens the suggestion for formation of a biotin-CO₂-G intermediate which, we suggest, must include partial decoupling of the carboxy- and ureido π -systems. Incomplete conjugation was not included in the planar, conjugated models Maggiora used to derive charge and overlap populations,³⁰ but such decoupling would increase the electrophilicity of the carboxy-carbonyl carbon, weaken the N'-C (carbonyl) bond, and hence facilitate CO₂ transfer to the acceptor molecule [reaction (2)]. The basicity of N' would also be enhanced, which is consistent with the suggestion^{3a} that N' might undergo protonation before CO₂ transfer to pyruvate.

We thank the Welch Foundation and the National Institutes of Health (H. K.) for financial support.

[6/2365 Received, 31st December, 1976]

¹⁹ W. L. Duax and D. A. Norton, 'Atlas of Steroid Structures,' vol. 1, Plenum, New York, 1975.

²⁰ C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, 1968, **24**, 13.

²¹ K. W. Wong, Ph.D. Thesis, Louisiana State University, Baton Rouge, Louisiana, 1975.

²² M. R. Truter, *Acta Cryst.*, 1967, **22**, 556.

²³ M. M. Elcombe and J. C. Taylor, *Acta Cryst.*, 1968, **A24**, 410.

²⁴ J. C. A. Boeyens, *Acta Cryst.*, 1968, **B24**, 199.

²⁵ J. Mitchell and J. C. A. Boeyens, *Acta Cryst.*, 1970, **B26**, 1121.

²⁶ P. M. Colman and E. H. Medlin, *Acta Cryst.*, 1970, **B26**, 1553.

²⁷ M. R. Truter and B. L. Vickery, *Acta Cryst.*, 1972, **B28**, 387.

²⁸ W. B. Wright and E. A. Meyers, *Cryst. Struct. Comm.*, 1973, **3**, 477.

²⁹ O. Kennard, D. G. Watson, F. H. Allen, N. W. Isaacs, W. D. S. Motherwell, R. C. Pettersen, and W. G. Town, 'Molecular Structures and Dimensions,' vol. A1, Oosthoek, Utrecht, 1972.

³⁰ G. M. Maggiora, *J. Theor. Biol.*, 1974, **43**, 51.