method B. The crude bright yellow cryst solid (12.5 g, 44%) was converted *via* method C to the tosylate (9.0 g, 58%), which in turn was allowed to react *via* method G to give 4.1 g (73%) of required product, mp 147–148°.

1-(2-Chloroethyl)-2-(4-methylstyryl)-5-nitroimidazole (53). Compd 13 (27.3 g, 0.1 mole) was stirred in 60 ml of DMF at room temp. $SOCl_2$ (7.5 ml, >0.1 mole) was added dropwise to the clear soln. A crystal mass formed after *ca.* 5 min, and, after standing for 1 hr, the crystals were filtered, washed with C_6H_6, and dried to give 22.5 g (77%) of product, mp 159–160°.

2-(4-Methylstyryl)-1-(2-morpholinoethyl)-5-nitroimidazole (52). Tosylate 31 (21.4 g, 0.05 mole) and 50 ml of morpholine were warmed on the steam bath for 2 hr. The dark brown soln was cooled and dild with 50 ml of C_6H_6, followed by 200 ml of Et_2O pptg out morpholine tosylate. The supernatant was C treated, evapd (*in vacuo*) to low vol, and treated with *n*-hexane to dissolve out morpholine. The resultant oily solid was dissolved in C_6H_6–Et_2O (75:25 v/v) and chromatographed on a silica gel column (500 g) ultimately yielding a yellow solid. Crystn from EtOAc–Et_2O gave 1.5 g (9%) of product, mp 125–126°.

5-Nitro-2-(4-tolylethynylene)-1-vinylimidazole (81). Tosylate 31 (71.3 g, 0.17 mole) was stirred in 500 ml of CCl_4, and 9.5 ml of bromine was added dropwise. The bromine color gradually faded, and the mixture was refluxed gently for 1 hr. After standing overnight at room temp, the cream-colored crystals were filtered to give 88.0 g (90%) of the required dibromo compound, mp 156–157°. A suspension of this dibromo compound (29.4 g, 0.05 mole) was stirred in 150 ml of DMSO. DBN (12.4 g, 0.1 mole) was added dropwise to the suspension maintaining the temp at 25–30°. The mixture was heated to 80° and maintained for 3 hr. The yellow-brown soln was cooled and added dropwise with stirring to 750 ml of ice H_2O. The cryst ppt which formed initially soon darkened and became sticky. The supernatant was decanted, and the residual solid was dissolved in $CHCl_3$, C treated, and evapd to give an oil which was extracted repeatedly with petr ether (60–80°) to give a bright yellow cryst solid (13.2 g, 61%) which was treated *via* method G to give 3.8 g (43%) of the required acetylenic product, mp 179–180°.

2-Methyl-5-nitro-1-vinylimidazole (5, R = $CH{=}CH_2$). 1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole (50 g, 0.29 mole) was treated by method C to yield 86.1 g (91%) of the tosylate, mp 153°, which was treated as in method G. After cooling to room temp for several hours, the dark brown reaction mix was filtered, and the filtrate was evapd to a vol of *ca.* 100 ml. The concentrate was dild with 500 ml of H_2O and extd with Et_2O (four 250-ml portions). The combined exts were C treated, dried over Na_2SO_4, and evapd to a vol of *ca.* 100 ml, and *n*-hexane was added carefully to ppt a reddish oil which was discarded. The extract was further dild with *n*-hexane to ppt 22.9 g (55%) of the desired product as pale yellow needles, mp 49–50°.

1-Ethyl-2-methyl-5-nitroimidazole (5, R = Et). A suspension of 127 g (1.0 mole) of 2-methyl-5-nitroimidazole in 200 ml of DMF containing 165 g of $(C_2H_5)_2SO_4$ (1.07 moles) was stirred and heated on a steam bath for 3 hr. The DMF was evapd *in vacuo*, and the residue was poured into H_2O when solid (starting material) sepd. This was filtered off, and the filtrate was brought to pH 5 when further solid separated. This was filtered off and added to first solid (total 50 g). The filtrate was basified with solid $NaHCO_3$ and extd with $CHCl_3$ (five 200-ml portions). The $CHCl_3$ extract was dried over $MgSO_4$, the solvent removed *in vacuo*, and the oily residue distd *in vacuo* to give 37.5 g (24%) of the required product, bp 110–112° (1.0 mm). This was identical (ir, nmr) with material prepared by the literature method.[10]

Acknowledgments. We thank Dr. M. D. Pittam, Mr. P. G. Robins, Mr. B. Ramsahye, and Miss F. C. Wright for the biological results. Dr. N. C. Franklin and Dr. D. M. Rackham are thanked for the nmr data and Mr. G. Maciak of the Lilly Research Laboratories, Indianapolis, Ind., for the microanalytical data on the compounds described in this paper.

References

(1) K. Maeda, T. Osato, and H. Umezawa, *J. Antibiot.*, 6A, 182 (1953).
(2) S. Nakamura, *Pharm. Bull.*, 3, 379 (1955).
(3) H. Horie, *J. Antibiot.*, 9, 168 (1956).
(4) C. Cosar, T. Jolon, and M. Bonazet, *Ann. Inst. Pasteur*, 96, 238 (1959).
(5) Antiparasitic Section, *Annu. Rep. Med. Chem.* (1965), and thereafter.
(6) G. P. Ellis, C. Epstein, C. Fitzmaurice, L. Goldberg, and G. H. Lord, *J. Pharm. Pharmacol.*, 16, 801 (1964).
(7) P. N. Giraldi, V. Mariotti, and I. de Carneri, *J. Med. Chem.*, 11, 66 (1968).
(8) Merck and Co., Inc., U. S. Patent 3,472,864 (Oct 14, 1969); Merck and Co., Inc., French Patent 1,423,701 (Aug 6, 1964).
(9) A. F. Cockerill, R. C. Harden, and D. N. B. Mallen, *J. Chem. Soc., Perkin Trans. 2*, in press.
(10) C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitisheff, and R. Vanpre, *Arzneim.-Forsch.*, 16, 23 (1966).
(11) K. Eiter and H. Oediger, *Justus Liebigs Ann. Chem.*, 682, 62 (1965).
(12) G. Kobrich and P. Buck in "Chemistry of Acetylenes," H. G. Viehe, Ed., Marcel Dekker, New York, N. Y., 1969, pp 100–101.
(13) H. C. Brown and G. Zweifel, *J. Amer. Chem. Soc.*, 83, 3834 (1961).
(14) M. Honigberg, *J. Parasitol.*, 47, 545 (1961).
(15) A. C. Cuckler, C. M. Malanga, and J. Conroy, *Amer. J. Trop. Med. Hyg.*, 19, 916 (1970).
(16) W. R. Jones, *Ann. Trop. Med. Parasitol.*, 40, 130 (1946).
(17) J. W. Reinertson and P. E. Thompson, *Proc. Soc. Exp. Biol. Med.*, 76, 518 (1951).
(18) F. Hawking, *Exp. Chemother.*, 1, Chapter 5 (1963).
(19) Rhone-Ponlenc S. A., Eire Patent 1058/64 (Oct 12, 1964).

Metabolism of 2-(4-Chlorophenyl)thiazol-4-ylacetic Acid (Fenclozic Acid) and Related Compounds by Microorganisms

Ralph Howe,* Ronald H. Moore, Balbir S. Rao, and Alan H. Wood

Imperial Chemical Industries Limited, Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, England.
Received January 26, 1972

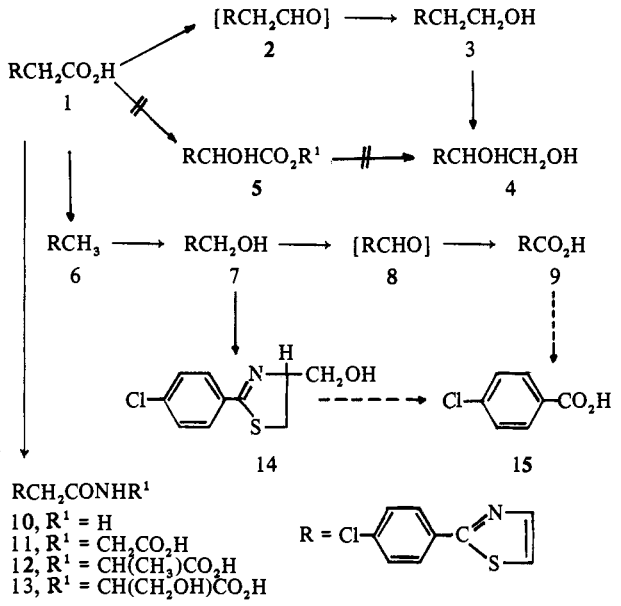
Eleven metabolites produced by microorganisms from the antiinflammatory agent fenclozic acid (1) differed from those produced by mammals. There was no overlap. Microorganisms preferred to attack the acetic acid side chain whereas mammals hydroxylated the 4-chlorophenyl ring. The alcohol metabolite (3) had similar antiinflammatory activity to fenclozic acid, and the amides 10–13 showed an interesting level of potency, but no metabolite was more potent. A novel metabolic α-hydroxylation of the alcohol 3 has been shown to occur with 100% stereospecificity.

2-(4-Chlorophenyl)thiazol-4-ylacetic acid (1) (fenclozic acid) is a potent antiinflammatory agent in rats, mice, and guinea pigs.[1-3] It has been evaluated in patients with rheumatoid arthritis, but was withdrawn when it was found to produce cholestatic jaundice in patients receiving a high initial dose.[4] The action of microorganisms on fenclozic acid has been studied in order to compare the metabolites with those produced by mammals.[5] Desired metabolites

could then perhaps be produced more readily in quantity by fermentation.[6] There was also the possibility that a biologically active metabolite might be produced, for there is a growing number of drugs whose biological activity is partly or completely due to a metabolite.[7] The method of screening organisms for their ability to metabolize **1** and the subsequent production, isolation, and identification of metabolites are dealt with in the Experimental Section.

The transformation products, **3**, **4**, **6**, **7**, **9–15**, obtained from fenclozic acid and proposed metabolic pathways, are shown in Scheme I. Organisms rarely produced a single

Scheme I



transformation product. Many organisms produced the same range of products, but in different relative amounts. Since this was primarily a screening and identification exercise, only a nominal attempt was made to control fermentations by altering inoculum size, medium, substrate concentration, timing of addition and harvesting, etc. Thus for a particular organism the distribution of products usually varied slightly from batch to batch. Nevertheless, sufficient material was obtained in this way to establish the identity of and the degree of interest in the products. By monitoring the sequence of events in flask or fermenter batches by tlc, and by offering various products and derivatives in turn to selected organisms, it was possible to discern the metabolic pathways shown in Scheme I. Organisms which carry out certain of the conversions are listed in Table I. Each metabolite listed was isolated and characterized chemically at least once. The methyl ester of fenclozic acid could be used as substrate in place of **1**, for it was converted readily to the acid **1** during the fermentations.

The first transformation product identified, and the one which was subsequently shown to be produced most frequently, was the alcohol **3**.[8,9] There have been recent reports of similar reductions of acids containing aromatic rings to the corresponding alcohols *via* aldehydes.[10] We have no evidence for the presumed aldehyde intermediate **2** in the metabolic sequence, and several attempts to prepare a synthetic sample were unsuccessful. The reduction process was reversible under fermentation conditions as some organisms were found to oxidize the alcohol **3** to fenclozic acid **1**. In some fermentations which were monitored by tlc, conversion of **1** to **3** and subsequent reappearance of **1** at the expense of **3** was observed.

Five organisms produced the diol **4** as a minor metabolite along with the alcohol **3**. Three of those five organisms tried would convert added **3** to **4**, suggesting that **3** was on the metabolic pathway from **1** to **4**. One organism, *Penicillium duclauxi*, which was unable to convert **1** to **3** under the fermentation conditions, but which could oxidize **3** to **1**, could also convert added **3** to **4**. There seems to be no precedent for this conversion of an alcohol to an α-glycol by microorganisms, although the production of α-glycols from olefins is well known.[11] Four of the organisms, ACC numbers 505, 2343, 2614, and 2895, which produced the

Table I

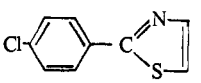
Organism	ACC No.[a]	1→3	1→4	1→7	1→10	3→1	3→4	7→9
Phycomycetes								
Mucor ramannianus Möller	647	C[b]		C				
Rhizopus sp	2471	C		I				
Zygorhyncus moelleri Vuill (IMI 135206)	545	I[c]			I			
Z. moelleri Vuill (IMI 135207)	544	C			C			
Ascomycetes								
Hendersonula toruloidea Nattrass (IMI 135205)	1757	C	C		I			
Hypomyces rosellus (Alb and Schw) Tul (CBS)	1408	C						
Fungi Imperfecti								
Botryodiplodia ananassae (Sacch) Petrak (IMI 80379)	2646	C						
Colletotrichum atramentarium (Berk & Br) Taubenh (IMI 95426)	1866	C	C		C			
C. coffeanum Noack (IMI 108201)	2614	I	C				C	
Lasiodiplodia theobromae (FPRL S22L)	2895	I	I	I		C	I	I
Macrophoma sugi Hara (IAM 4138)	2343	I	C		C		C	
Penicillium canescens Sopp (IMI 135210)	1260	C		C	I			
P. canescens Sopp	879			I	I			
P. canescens Sopp	507			C				
P. duclauxi Delacroix	505			I		I	I	
P. hirayamae Udagawa (IMI 78255)	1397	C		C				
Trichoderma harzianum (Trichoderma sp) (IMI 135208)	1900	I						
T. koningii (Oud) (IMI 135211)	985							
Trichothecium roseum (Pers) Link ex Fr	39				I			
Bacteria								
Unidentified (NCIB 10330)	2237	I						
Xanthomonas malvacearum (Erw Smith) Dowson	2312			C				

[a] I.C.I. internal reference number for organism. [b] C, product identified by tlc in at least 2 solvent systems. [c] I, product identified chemically.

diol **4**, did not metabolize it when it was added as substrate. An alternative route from the acid **1** to the diol **4** is possible in which α-hydroxylation precedes reduction of the carboxyl group. We have found no evidence to suggest that the hydroxy acid **5** (R^1 = H) is formed from **1** or that it can be reduced to the diol **4**. The acetonide of the diol **4** isolated from one *Lasiodiplodia theobromae* fermentation is assumed to be an artefact. The diol **4** isolated from *L. theobromae* transformations was optically active, an aspect which is considered later.

A further alcohol metabolite, which was produced by several organisms, was identified as 7. This was presumed to be formed by hydroxylation of the intermediate **6**,[12] formed from **1** by decarboxylation. Fenclozic acid decarboxylates spontaneously in aqueous solution.[13] Decarboxylation proceeds slowly at pH 6-7 but is more rapid at pH 2. The decarboxylated material was present in every fermentation of fenclozic acid, and in the substrate control without the organism. Certainly some, and possibly all, of the intermediate **6** is formed nonenzymically in amounts related to the pH developed in the medium by the growing organism. Hydroxylation of methyl groups attached to a heterocyclic ring is well known.[6,14] One organism, *Penicillium duclauxi*, once produced a small amount of the reduced thiazol-2-ine alcohol **14**, but all subsequent attempts to obtain more gave the thiazole alcohol 7. The alcohol 7 was oxidized to the corresponding acid **9** by *L. theobromae*, and tlc evidence was obtained for the conversion of the supposed intermediate aldehyde 8 to the acid **9** by this organism, and also by ACC numbers 505, 2343, and 2614. Chromatographic evidence also showed that **9** formed from 7 could be reduced to 7 during the fermentation with *L. theobromae*, just as observed with fenclozic acid.

Apart from *P. duclauxi* mentioned above, only one other organism produced evidence for attack on the thiazole ring. A small amount of 4-chlorobenzoic acid, which is not a known natural product, and which was not detected in control fermentations without added fenclozic acid, was obtained from a *Mucor ramannianus* fermentation. 4-Hydroxybenzoic acid, isolated from both *M. ramannianus* and *Rhizopus sp.* fermentations of fenclozic acid, was present in the control fermentations and has previously been isolated in these laboratories from a fermentation using the former organism.† It is possible, however, that some of the phenolic acid came *via* 2-(4-hydroxyphenyl)thiazol-4-ylacetic acid, a known mammalian metabolite of fenclozic acid.[5] Surprisingly, we were unable to obtain definite evidence for attack on the benzene ring, in spite of the known ability of microorganisms to do so,[15] and the production of phenolic compounds from fenclozic acid by mammals.[5] Attack on the side chain was always preferred. Neither fenclozic acid nor 2-(4-chlorophenyl)thiazole **16**,[16] which lacks a side



16

chain, was attacked by five *Aspergillus niger* strains which included two‡ known to produce phenols from chlorophenoxyacetic acids. Phenol formation was not induced by prior exposure of *A. niger* to 2,4-dichlorophenoxyacetic acid.

†Personal communication from Dr. D. F. Jones.

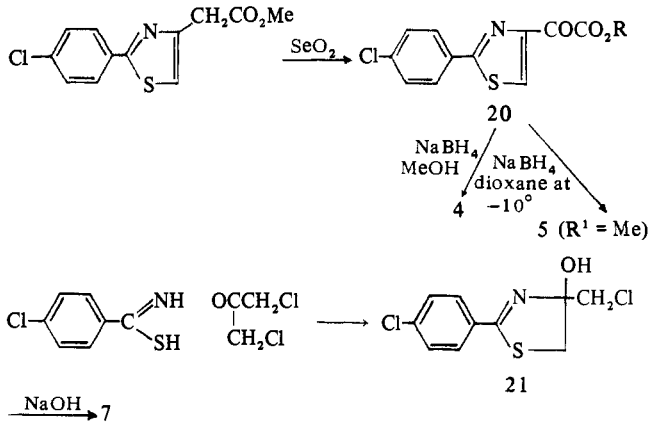
‡CMI No. 31283 and *Aspergillus niger* van Tiegh Mulder strain. The latter organism was kindly supplied by Dr. D. Woodcock of the University of Bristol, Department of Agriculture and Horticulture, Research Station, Long Ashton, Bristol.[17]

Several organisms converted fenclozic acid to the corresponding amide **10**,[18] and one, *M. ramannianus*, formed conjugates with glycine (**11**),[18] L-alanine (**12**), and L-serine (**13**). This seems to be a characteristic of the organism for it has subsequently been shown to produce similar conjugates from mycophenolic acid.[19] *Bacillus megaterium* has been reported to convert indolepropionic acid to alanine, serine, and threonine conjugates.[20]

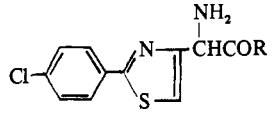
17 and 18, R as in Scheme I 19

2-(4-Chlorophenyl)thiazol-4-ylethylamine (**17**)[21] was added to a fermentation of the glycol-producing organism *L. theobromae* to see whether a β-hydroxyamine would be formed. The major transformation product was the *N*-acetyl derivative **18**. Fenclozic acid was also produced, along with the expected traces of the alcohol 3 and the diol **4**. The amine **17** was rapidly converted to fenclozic acid in rats.§ Biochemical precedent is provided by the conversion of tryptamine to indoleacetic acid and tryptophol by the rat[22] and by *A. niger*.[23] An analog of fenclozic acid, methyl 2-(4-chlorophenyl)-6-methoxypyrimidin-4-ylacetate (**19**, R = CO_2Me),[24] was converted to the corresponding alcohol (**19**, R = CH_2OH)[8] and amide (**19**, R = $CONH_2$) by *Colletotrichum coffeanum*. The methyl ester was used as substrate because the acid decarboxylates more readily than does fenclozic acid.

Synthetic Chemistry. The racemic diol 4 and the glycollic ester **5** (R^1 = Me) were prepared *via* the glyoxylic ester **20** (R = Me). The alcohol 7 was obtained by the action of alkali on the thiazol-2-ine **21**, formed from 4-chlorothiobenzamide and *sym*-dichloroacetone. Oxidation of 7 with Jones reagent gave a mixture of the aldehyde 8 and the acid **9**. The L-alanine and L-serine conjugates **12** and **13** were prepared by the DCCI coupling procedure.



Incidental to the main theme of this work, the oxime of the glyoxylic ester **20** (R = Me) was reduced by Zn-HOAc to the substituted glycine ester **22** (R = OMe) and by Zn-



22

HOAc + NH_4OAc to the glycinamide **22** ($R = NH_2$).

Stereochemistry of Hydroxylation of 3. It was of interest

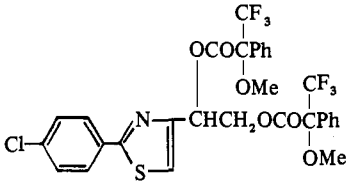
§Dr. D. S. Platt, personal communication.

Table II

Compound	Dose, mg/kg	% inhibition Day 3	% inhibition Day 13
1	50		65
	20[a]	42	40
3	20	39	51
4 (racemic)	100	14	20
	25	10	11
5 (R^1 = H)	50	6	0
7	100	6	17
9	100	34	19
10	200	56	60
	20	44	20
11	100	46	60
	20	44	31
12 (Me ester)	100	55	72
	20	35	41
13 (Me ester)	100	41	36
	20	37	24
16	100	17	0
20	200	46	37
	50	0	17
20 (oxime)	100	8	0
22 (R = OMe)	100	41	10
22 (R = NH_2)	100	11	41

[a] Average results from 39 rats.

to know the optical purity of the (−)-diol **4** produced by *L. theobromae*. This was determined using both proton and fluorine magnetic resonance spectra. Racemic diol **4** was converted to a mixture of two diastereoisomeric diesters[25] **23** from which contaminants were removed by prep tlc. The



23

mixture (2 spots), which was not otherwise separated, showed clear signals at τ 6.07 and 6.33 (CCl_4) due to the 5 H of the thiazole in the two diastereoisomers. The fermentation (−)-diol **4** was treated in the same way to give a single spot which showed the signal at τ 6.07 but completely lacked a signal at τ 6.33. The diester mixture from the racemic diol showed two broad signals (CCl_4) at 72.6 and 72.8 ppm ($CFCl_3$ standard) from the CF_3 groups of the secondary alcohol esters, and a broad signal at 72.9 ppm from the CF_3 groups of the primary alcohol esters. Integration showed a 1:1 ratio of diastereoisomers. The diester from the fermentation (−)-diol showed two broad signals at 72.8 and 72.9 ppm; there was no signal at 72.6 ppm. Thus the material is judged to be optically pure. The absolute configuration remains in doubt.

Biological Results. The new compounds were tested for inhibition of adjuvant-induced arthritis in rats (developing) by Dr. B. B. Newbould[2] and his coworkers.

Groups of 3 rats were dosed orally with the test compounds and, on the day after dosing, an injection of dead tubercle bacilli was given into the foot pad. The test compound was then dosed orally each day until the 13th day after the injection of the tubercle bacilli. The thickness of the injected foot was measured 3 days and 13 days after injection. The percentage inhibition of the increase in thickness of the foot was then calculated. The results are shown in Table II. The alcohol **3** was of particular interest, being equiactive with fenclozic acid **1**, to which it is metabolized by rats.[§] The amides **10–13** showed an interesting level of potency, possibly due to hydrolysis to fenclozic acid.

Experimental Section

Tlc was carried out using SiO_2 gel GF-254 plates developed in one or more of the solvent systems (a) EtOAc; (b) 40% HCO_2H-EtOAc, 1:99; (c) (C_6H_6–dioxane–HOAc, 90:25:4. Products were detected by their blue fluorescence in uv light (350 mμ), particularly after spraying with H_2SO_4–MeOH, 1:99, and as charred spots after spraying with chromic acid and heating. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Screening for Metabolites. Initial screening was carried out in shaken test-tube cultures of microorganisms chosen at random from our collection. The substrate (100 μg) in Me_2CO (0.1 ml) was added to a shaken test-tube culture (3 ml) of the organism which had been grown at 25° for 48 hr. The medium consisted of corn steep liquor (0.3% solids), peptone 2%, dextrose 5%, minor element concentrate[26] 0.1% in deionized H_2O, adjusted to pH 4.5 and then autoclaved at 120° and 15 psi for 15 min. The cultures plus substrate were incubated for 3–4 days and then extd at pH 3 with EtOAc. The exts were compared by tlc with control exts from the organisms alone and the substrate alone in the nutrient medium. Substrate–organism combinations which produced new products were scaled up to shake-flask cultures and/or stirred fermenters. Over 500 organisms were examined, including fungi (Phycomycetes, Ascomycetes, and Fungi Imperfecti), bacteria, Streptomycetes, algae, and a protozoon.

Isolation of Metabolites. In a typical expt the contents of shake flasks or fermenter were extd with EtOAc at pH 2 and pH 10. The exts were evapd to dryness, and the residues were combined. The mixt was dissolved in Et_2O and sepd conventionally into acid, phenolic, basic, and neutral fractions. These exts were then compared by tlc with corresponding fractions from the organism and the substrate controls. Metabolites were isolated and identified by conventional procedures and were then used as standards in subsequent screening expts.

Metabolism of 2-(4-Chlorophenyl)thiazol-4-ylacetic Acid (1). (a) By *C. coffeanum* Noack **108201**. Four 5-l. stirred fermentations of substrate 1[3] (1 g) were carried out for 23 hr in a medium containing glucose (50 g), tartaric acid (2.6 g), ammonium tartrate (2.6 g), $(NH_4)_2HPO_4$ (0.4 g), K_2CO_3 (0.4 g), $MgCO_3$ (0.26 g), $(NH_4)_2SO_3$ (0.16 g), $ZnSO_4 \cdot 7H_2O$ (0.046 g), and $FeSO_4 \cdot 7H_2O$ (0.046 g) per 1 l. of distilled H_2O. The neutral fraction (0.85 g), mainly one product, R_f 0.6 (system b), was stirred with a mixt of Et_2O and petr ether (bp 40–60°) (1:1) and then filtered to remove insol material. The filtrate was evapd to dryness, and the residue was extd with hot petr ether (bp 40–60°). The ext gave 3, mp 65–66° (0.3 g). *Anal.* ($C_{11}H_{10}ClNOS$) C, H, Cl, N, S.

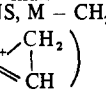
(b) By *L. theobromae* **F.P.R.L. S22L.** An 80-l. stirred fermentation of substrate 1 (5 g) was carried out for 173 hr in a medium containing glucose monohydrate (50 g), ammonium tartrate (10 g), KH_2PO_4 (2.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (1.0 mg), $CuSO_4 \cdot 5H_2O$ (0.15 mg), $ZnSO_4 \cdot 7H_2O$ (1.0 mg), $MnSO_4 \cdot 4H_2O$ (0.1 mg), and K_2MoO_4 (0.1 mg) per 1 l. of distilled H_2O. The neutral fraction (2.1 g) was sepd into (a) material sol in petr ether (bp 40–60°) (1.5 g), (b) material sol in $MgCO_3$ (0.5 g), and (c) insol residue. Fraction b gave (−)-4, mp 120–121° (100 mg) from Me_2CO, $[\alpha]^{23}D$ −61.4° (*c* 1.01, EtOH), R_f 0.32 (system a). *Anal.* ($C_{11}H_{10}ClNO_2S$) C, H, N. The mother liquors from the above crystn of the diol 4 contained the diol 4, R_f 0.32 (system a), the alcohol **3**, R_f 0.46, and an unknown, R_f 0.77 (yellow spot with 2,4-DNP). The unknown (15 mg), obtained as a gum by prep tlc, was the acetonide of **4**, *m/e* 295 ($C_{14}H_{14}ClNO_2S$) identical by tlc and mass spec with synthetic racemate.

Fraction a was chromatographed on SiO_2 (40 g) and eluted with petr ether (bp 60–80°) followed by increasing proportions of C_6H_6 in petr ether, and then increasing proportions of $CHCl_3$ in C_6H_6 up to 70% $CHCl_3$ by vol. These solvent systems eluted fatty materials of $R_f > 0.7$ (system a). Elution with $CHCl_3$ gave a fraction (330 mg) which contained the alcohol **3**, R_f 0.47, and an unknown, R_f 0.52 (system a). The alcohol **3** was isolated by prep tlc, mmp 66° (75 mg). The unknown, R_f 0.52, isolated similarly proved to be 7, mmp 124–125°, from Et_2O–petr ether (bp 40–60°) (15 mg).

(c) By *Hendersonula toruloidea* **Nattrass IMI 135205.** A 5-l. fermentation of substrate 1 (250 mg) was carried out for 367 hr. The neutral fraction (254 mg) contained the alcohol 3, R_f 0.46, the diol 4, R_f 0.32, and a new material, R_f 0.17 (system a), iso-

lated by prep tlc and shown to be the amide **10** (5 mg), mmp 171–172°, *m/e* 252.[18]

(d) By *Mucor ramannianus* Möller. An 80-l. fermentation of substrate 1 (5 g) was carried out for 70 hr. The ext was stirred with EtOAc–Et_2O (3:2), the solid which sepd was isolated, and the filtrate was retained. The solid was recrystd from EtOH to give material, mp 228–230°, R_f 0.38, contaminated with materials of R_f 0.44 and R_f 0.22 (system b). Treatment of the solid, mp 228–230° with CH_2N_2 gave a methyl ester which was chromatographed on SiO_2. Elution with $CHCl_3$–petr ether (bp 60–80°) (1:1) gave an ester (520 mg), R_f 0.5, containing material of R_f 0.55 (system b). Final purification by prep tlc gave the methyl ester of 11, mmp 144–145°[18] from MeOH (220 mg). The filtrate retained above was sepd into (a) a fraction sol in 5% $NaHCO_3$ (2.3 g), (b) a fraction sol in 1 *N* NaOH (0.78 g), and (c) a neutral fraction (20.6 g). Fraction a contained materials of R_f 0.22 (the serine conjugate 13), R_f 0.38 (the glycine conjugate 11), R_f 0.44 (the alanine conjugate **12**), R_f 0.51 (4-chlorobenzoic acid 15), and R_f 0.63 (4-hydroxybenzoic acid) (system b). It was treated with CH_2N_2 and then sepd by chromatography on a SiO_2 column followed by repeated prep tlc. There was thus obtained the methyl ester of **12** (30 mg), R_f 0.55 (trace impurity R_f 0.5, the ester of 11) (system b), mmp 122–123° from MeOH, $[\alpha]^{21}D$ −25° (*c* 0.94, MeOH), and the methyl ester of 13 (40 mg), R_f 0.46 (system b), mmp 153–154° $[\alpha]^{21}D$ +1° (*c* 1.37, MeOH). Fraction b sepd by prep tlc gave 4-chlorobenzoic acid R_f 0.51 (system b), mmp 241° (5 mg), and 4-hydroxybenzoic acid, R_f 0.63 (system b), mmp 210° (**10** mg). Fraction c, mainly fatty material, contained the alcohol **3**, R_f 0.46 (system a).

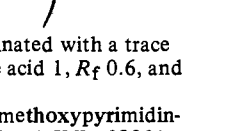
(e) By *Penicillium duclauxi* Delacroix. A shake-flask fermentation of 1 (60 mg) was carried out for 240 hr. The ext gave a basic fraction (13 mg) which solidified and gave 14, mp 101–102° (4 mg) from petr ether (bp 40–60°), R_f 0.5 (system b), λ_{max} 250.7 (ε 21,900), *m/e* 227 ($C_{10}H_{10}ClNOS$), 196 (C_9H_7ClNS, M − CH_2OH), 155 ($ClC_6H_4C{\equiv}S^+$), 138 ($ClC_6H_4C{\equiv}N^+H$), 59 

Several subsequent experiments on a larger scale aimed at producing 14 in quantity gave the related thiazole 7, rather than 14.

Metabolism of **2-(4-Chlorophenyl)thiazol-4-ylethanol (3).** (a) By *L. theobromae* F.P.R.L. **S22L.** A 5-l. stirred fermentation of substrate **3**[8] (0.25 g) was carried out for 76 hr as in b in the previous expt. The neutral fraction (460 mg) was chromatographed on Al_2O_3 and eluted with petr ether (bp 60–80°), and then increasing proportions of EtOAc in petr ether. Elution with 50% EtOAc gave the diol 4, mmp 120–121° (80 mg). The acid fraction (166 mg) contained the acid 1, R_f 0.65 (system b).

(b) By *P. duclauxi* Delacroix. A 5-l. stirred fermentation of substrate 3 (0.25 g) was carried out for 105 hr. The neutral fraction (650 mg) gave the diol 4 (35 mg), and the acid fraction (166 mg) gave the acid 1, mmp 151° (25 mg) by prep tlc.

Metabolism of 2-(4-Chlorophenyl)-4-hydroxymethylthiazole (7). By *L. theobromae* F.P.R.L. **S22L.** A 5-l. stirred fermentation of substrate 7 (0.25 g) was carried out for 65 hr. The acid fraction (182 mg) was shown by tlc (system c) to be almost entirely 2-(4-chlorphenyl)thiazole-4-carboxylic acid (9), mmp 188–190° from EtOAc. The neutral fraction contained no starting material 7 and none of the related aldehyde **8.**

Metabolism of 2-(4-Chlorophenyl)thiazol-4-ylethylamine (17). By *L. theobromae* F.P.R.L. **S22L.** A 5-l. stirred fermentation of 17 oxalate (0.25 g) was carried out for 65 hr. The neutral fraction (153 mg) contd a major new product, R_f 0.35 (system c), and traces of the alcohol **3** and the diol 4. The new product, isold by prep tlc was 18, mmp 149–150° (35 mg) from Et_2O, *m/e* 280, 221 (M − CH_3CONH_2, 174.4*), 209  The basic fraction (22 mg) was predominantly **18**, contaminated with a trace of 17. The acid fraction (79 mg) consisted of the acid 1, R_f 0.6, and **18**, R_f 0.35 (system c).

Metabolism of Methyl 2-(4-Chlorophenyl)-6-methoxypyrimidin-4-ylacetate (19, R = CO_2Me). By *C. coffeanum* Noack **IMI 108201.** The culture medium contained glucose (10 g), ammonium tartrate (2 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (1.0 mg), $CuSO_4 \cdot 5H_2O$ (0.15 mg), $ZnSO_4 \cdot 7H_2O$ (1.0 mg), $MnSO_4 \cdot 4H_2O$ (0.1 mg), and K_2MoO_4 (0.1 mg) per liter of distd H_2O. The resulting soln was adjusted to pH 5.5 with 10 *N* NaOH. Conical flasks (25) (500 ml) contg medium (200 ml) and plugged with cotton wool were sterilized at 120° and 15 psi for 20 min. The flasks were inoculated and then incubated at 25° on a rotary shaker. After 48 hr, a soln of 19 (R = CO_2Me)[24] (0.01 g) in Me_2CO (1.5 ml) was added to each flask and 240 hr later the fermentation was stopped. The neutral fraction (0.36 g) was sepd by prep tlc [EtOAc–C_6H_6 (1:1)]. The band R_f 0.48–0.60 was eluted with EtOH and gave 19 (R = CH_2OH)[8] (36 mg), mmp 101–102° from C_6H_{12}. The band R_f 0.1–0.2 was eluted with EtOH and gave 19 (R = $CONH_2$) (65 mg), mmp 185–186° from C_6H_6.

Methyl 2-(4-Chlorophenyl)thiazol-4-ylglyoxylate (20, R = Me). The Me ester of 1 (54 g), SeO_2 (26 g), and xylene (300 ml) were heated under reflux for 8 hr and then filtered. The filtrate was evapd to dryness, and the residue was extrd with hot petr ether (bp 60–80°) to remove starting material. The insol residue gave **20** (R = Me), mp 131–132° from Me_2CO–C_6H_{12} (30 g, 53%). *Anal.* ($C_{12}H_8ClNO_3S$) C, H, N. The oxime of **20** (R = Me) had mp 159–160° from EtOAc. *Anal.* ($C_{12}H_9ClN_2O_3S$) C, H, N.

2-(4-Chlorophenyl)thiazol-4-ylglyoxylic Acid (20, R = H). **20** (R = Me) (1 g) and NaOH (0.2 g) in MeOH (10 ml) were heated under reflux for 30 min, and then the soln was poured into H_2O and extd with Et_2O. The aqueous layer was acidified with 2 *N* HCl and then extd with Et_2O. This ext gave **20** (R = H), mp 224–225° from MeOH–H_2O. *Anal.* ($C_{11}H_6ClNO_3S$) C, H, N.

(±)-2-(4-Chlorophenyl)thiazol-4-ylethane-1,2-diol (4). $NaBH_4$ (400 mg) was added during 10 min to a soln of **20** (R = Me) (560 mg) in MeOH (50 ml). After 1 hr the MeOH was evapd, the residue was shaken with Et_2O and H_2O, and the Et_2O ext was washed with aqueous $NaHCO_3$. The ext gave (±)-4, mp 114° from Me_2CO (500 mg, 90%), ir, nmr, and mass spec were the same as those of (−)-4, R_f 0.32 (system a). *Anal.* ($C_{11}H_{10}ClNO_2S$) C, H, Cl, N; S: calcd. 12.5; found, 13.0. The acetonide, prepd by stirring 4 (125 mg) in Me_2CO (6 ml) with $HClO_4$ (2 drops) for 22 hr, was isold by prep tlc (system a), R_f 0.77, *m/e* 295.

Methyl 2-(4-Chlorophenyl)thiazol-4-ylglycollate (5, R^1 = Me). $NaBH_4$ (0.8 g) was added to a soln of **20** (R = Me) (6 g) in dioxane (80 ml) at −10°. After 2 hr, H_2O was added and the soln was extd with Et_2O. The Et_2O ext gave a gum which was extd with hot petr ether (bp 60–80°). This ext gave 5 (R^1 = Me), mp 95° from Et_2O–petr ether (bp 60–80°). *Anal.* ($C_{12}H_{10}ClNO_3S$) C, H, N. The corresponding acid (5, R^1 = H), mp 158–159° from $CHCl_3$, was obtained by alkaline hydrolysis of the ester as for **20** (R = H). *Anal.* ($C_{11}H_8ClNO_3S$) H, N; C: calcd, 49.0; found, 48.5.

4-Chloromethyl-2-(4-chlorophenyl)-4-hydroxythiazol-2-ine (21). *sym*-Dichloroacetone (10 g) in Me_2CO (100 ml) was added to 4-chlorothiobenzamide (13.5 g) in Me_2CO (150 ml) at room temp. After 24 hr, the solid 21 (5.2 g) was isolated by filtration and a further crop (7 g) obtained from the mother liquors, mp 154–156° from Et_2O–petr ether (bp 60–80°). *Anal.* ($C_{10}H_8Cl_2NOS$) C, H, N.

2-(4-Chlorophenyl)-4-hydroxymethylthiazole (7). 21 (12 g) was dissolved in MeOH (360 ml) and 12 *N* NaOH (6.5 ml) was added. After 20 min, the MeOH was evapd and the organic material was isolated by Et_2O extn. The ext gave 7, mp 125–126° from Et_2O (9 g, 51%). *Anal.* ($C_{10}H_8ClNOS$) C, H, Cl, N, S.

2-(4-Chlorophenyl)thiazole-4-carboxylic Acid (9) and 2-(4-Chlorophenyl)-4-formylthiazole (8). Jones reagent (13 ml) was added to a soln of 7 (6.6 g) in Me_2CO (250 ml) at room temp. After 1 hr, the Me_2CO was evapd, H_2O was added, and the organic material was extd into Et_2O. The Et_2O ext was washed with 1 *N* NaOH to remove acidic material. The Et_2O ext gave **8**, mp 122–123° from EtOAc. *Anal.* ($C_{10}H_6ClNOS$) C, H, N. The NaOH washings gave 9 after acidification and Et_2O extn, mp 189–190° from EtOAc. *Anal.* ($C_{10}H_6ClNO_2S$) C, H, N.

L-(−)-Methyl 2-α-[2-(4-Chlorophenyl)thiazol-4-yl] acetamidopropionate (Me Ester of 12). DCC (10 g) in $CHCl_3$ (100 ml) was added to 1 (12 g) in $CHCl_3$ (300 ml) at 0° and to the soln was added a freshly mixed soln of the Me ester of L-alanine·HCl (6 g) and Et_3N (4.5 g) in $CHCl_3$ (80 ml). The mixt was stirred at 0° for 3 hr and then filtered to give a residual solid (5.5 g). The filtrate was evapd to give further solid (26 g). The combined solids were chromatographed on SiO_2 (500 g) and eluted with petr ether (bp 60–80°) containing increasing proportions of Me_2CO. Solvent containing 15% Me_2CO eluted *N*-2-(4-chlorophenyl)thiazol-4-ylacetyl-*N,N'*-dicyclohexylurea, mp 154° from Me_2CO–petr ether (bp 60–80°). *Anal.* ($C_{24}H_{30}ClN_3O_2S$) C, H, N. Me_2CO eluted the Me ester of **12**, mp 123–124° from MeOH, $[\alpha]^{21}D$ −31.7° (*c* 1.8, MeOH). *Anal.* ($C_{15}H_{15}ClN_2O_3S$) H, Cl, N, S; C: calcd, 53.2; found, 53.7.

L-2-α-[2-(4-Chlorophenyl)thiazol-4-yl]acetamidopropionic Acid (12). The above ester (0.1 g) in 1 *N* NaOH (6.1 ml) and MeOH (2 ml) was kept at room temp for 2 days and then extd with Et_2O. The aqueous layer was acidified and then extd with Et_2O to give 12, mp 160–162°, R_f 0.44 (system b), τ ($CDCl_3$ and DMSO-d_6) 2.08 (AA^1 of AA^1BB^1, Ar-H, 2), 2.6 (BB^1 of AA^1BB^1, Ar-H, 2), 2.80 (s,

5 H of thiazole, 1), 5.5 (quintet, $CHCH_3$, 1), 6.2 (s, CH_2CO, 2), 8.6 (d, $CHCH_3$, 3).

L-(+)-Methyl 2-α-[2-(4-Chlorophenyl)thiazol-4-yl]acetamido-3-hydroxypropionate (Me Ester of 13). DCC (5 g) in $CHCl_3$ (50 ml) was added to 1 (6 g) in $CHCl_3$ (150 ml) at 0° and to the soln was added a freshly mixed soln of the Me ester of L-serine·HCl (3.68 g) and Et_3N (2.5 g) in $CHCl_3$ (40 ml). The mixt was stirred at 0° for 3 hr and then filtered to remove dicyclohexylurea. The filtrate was evapd and the residue was chromatographed on SiO_2. Elution with petr ether (bp 60–80°) and then with $CHCl_3$ gave unwanted material. The product was eluted with MeOH, mp 152°, $[\alpha]^{21}D$ +2.8° (*c* 1.36, MeOH), τ ($CDCl_3$) 2.3 (broad, NH, 1), 2.15 (AA^1 of AA^1BB^1, Ar-H, 2), 2.65 (BB^1 of AA^1BB^1, Ar-H, 2), 2.94 (s, 5 H of thiazole, 1), 5.4 (m, $CHCH_3$, 1), 6.12 (m, CH_2OH, 2), 6.28 (s, CH_2CO, 2), 6.32 (s, OCH_3, 3), 7.3 (broad, OH, 1).

α-[2-(4-Chlorophenyl)-6-methoxypyrimid-4-yl]acetamide (19, R = $CONH_2$). A soln of 19 (R = CO_2Me)[24] (0.9 g) in MeOH (10 ml) was treated with NH_4OH (0.880, 70 ml), kept at room temp overnight, and then heated at 100° for 30 min. Solid 19 (R = $CONH_2$) sepd, mp 185–186° from C_6H_6. *Anal.* ($C_{13}H_{12}ClN_3O_2$) C, H, N.

Methyl α-[2-(4-Chlorophenyl)thiazol-4-yl]glycinate Hydrochloride (22, R = OMe). The oxime of **20** (R = Me) (4 g) and Zn dust (9 g) in AcOH (70 ml) and H_2O (5 ml) were kept at room temp for 20 hr. The mixt was filtered, and the residual solid was washed with H_2O. The filtrate was neutralized with $NaHCO_3$ and then extd with EtOAc. The ext gave a gum which was dissolved in Et_2O. A little solid was removed by filtration and then ethereal HCl was added to the filtrate. The HCl of **22** (R = OMe) sepd, mp 202–203° from MeOH–EtOAc. *Anal.* ($C_{12}H_{12}Cl_2N_2O_2S\cdot 0.5H_2O$) C, H, N.

α-[2-(4-Chlorophenyl)thiazol-4-yl]glycinamide Hydrochloride (22, R = NH_2). The oxime of **20** (R = Me) (5 g), Zn dust (15 g), and NH_4OAc (5 g) in NH_4OH (0.88, 300 ml) and EtOH (50 ml) were heated under reflux for 3 hr. The cooled soln was filtered, the residue was washed with EtOH, and then the filtrate was evapd. The residue was extd with hot EtOAc (300 ml). The small amount of solid which sepd on concentrating and cooling this ext was removed by filtration. Ethereal HCl was added to the filtrate. The HCl of **22** (R = NH_2) sepd, mp 282–284° from MeOH–EtOAc. *Anal.* ($C_{11}H_{11}Cl_2N_3OS$) C, H, N.

α-[2-(4-Chlorophenyl)thiazol-4-yl]glycine (22, R = OH). A soln of **22** (R = NH_2) (60 mg) in 3 *N* HCl (3 ml) and EtOH (3 ml) was heated at 100° for 75 min and then the EtOH was evapd. The soln was made alkaline and filtered, and the filtrate made just acid. **22** (R = OH) sepd, mp 174–175° from H_2O. *Anal.* ($C_{11}H_9ClN_2O_2S$) C, H, N.

***N*-[2-(4-Chlorophenyl)thiazol-4-ylethyl]acetamide (18).** 17 (0.25 g), Ac_2O (2 ml), and 1 drop of concd H_2SO_4 were heated at 100° for 30 min. The soln was poured into H_2O, made alkaline, and extd with $CHCl_3$. The ext gave 18, mp 149–150° from EtOAc. *Anal.* ($C_{13}H_{13}ClN_2OS\cdot H_2O$) C, H, N.

(±)- and (−)-2-(4-Chlorophenyl)thiazol-4-ylethane-1,2-diol Di-(*S*)-α-methoxy-α-trifluoromethylphenylacetates (23). Enantiomerically pure, distilled *S*-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (0.1612 g, 0.65 mmole) was added to racemic 4 (0.077 g, 0.3 mmole) in dry C_5H_5N (1 ml). The mixt was heated at 45° for 1 hr, kept at room temp overnight, and then treated with H_2O and Et_2O. The Et_2O exts were washed (dil HCl, H_2O, satd Na_2CO_3), dried, and evapd to give an oil (0.189 g). This was purified by prep tlc (Et_2O–C_6H_{14}, 3:7) and the two bands at R_f's 0.50 and 0.48 were collected together (0.129 g) and analyzed by nmr (see Discussion).

In a similar manner (−)-**4** (0.044 g) gave the corresponding diester as an oil (0.060 g), R_f 0.48, which was analyzed by nmr.

Acknowledgments. We thank Dr. G. R. Bedford and Mr. D. Greatbanks, who obtained and discussed the nmr spectra, and Mr. M. J. Rix, who discussed the mass spectra.

References

(1) W. Hepworth, B. B. Newbould, D. S. Platt, and G. J. Stacey, *Nature (London)*, **221**, 582 (1969).
(2) B. B. Newbould, *Brit. J. Pharmacol.*, **35**, 487 (1969).
(3) W. Hepworth and G. J. Stacey, British Patent 1,099,389 (1968)
(4) *Lancet*, 1, 662 (1970).
(5) D. M. Foulkes, *Nature (London)*, **221**, 582 (1969); *J. Pharm. Exp. Ther.*, **172**, 115 (1970).
(6) P. B. Hamilton, D. Rosi, G. P. Peruzzotti, and E. D. Nielson, *Appl. Microbiol.*, 17, 237 (1969).
(7) P. A. Bond and R. Howe, *Biochem. Pharmacol.*, 16, 1261 (1967); G. R. Zins, *J. Pharm. Exp. Ther.*, **150**, 109 (1965); D. L. Smith, A. A. Forist, and G. C. Gerritsen, *ibid.*, **150**, 316 (1965); D. Rosi, G. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele, and S. Archer, *Nature (London)*, **208**, 1005 (1965); A. H. M. Raeymaekers, F. T. N. Allewijn, J. Vandenberk, P. J. A. Demoen, T. T. T. Van Offenwert, and P. A. J. Janssen, *J. Med. Chem.*, 9, 545 (1966).
(8) J. Hutton and G. J. Stacey, British Patent 1,180,268 (1970).
(9) R. Howe, R. H. Moore, B. S. Rao, and A. H. Wood, British Patent 1,183,850 (1970).
(10) G. G. Gross, K. H. Bolkart, and M. H. Zenk, *Biochem. Biophys. Res. Commun.*, **32**, 173 (1968); G. G. Gross and M. H. Zenk, *Eur. J. Biochem.*, **8**, 413, 420 (1969); T. K. Kirk, J. M. Harkin, and E. B. Cowling, *Biochem. Biophys. Acta*, 165, 145 (1968).
(11) E. J. McKenna and R. E. Kallio, *Annu. Rev. Microbiol.*, 19, 183 (1965).
(12) J. Eckenstein, E. Brogle, E. Sorkin, and H. Erlenmeyer, *Helv. Chim. Acta*, **33**, 1353 (1950).
(13) P. J. Taylor, *J. Chem. Soc. B*, in press.
(14) D. Rosi, C. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele B. F. Tullar, and S. Archer, *J. Med. Chem.*, **10**, 867 (1967).
(15) D. T. Gibson, *Science*, 161, 1093 (1968).
(16) H. Erlenmeyer, J. Eckenstein, E. Sorkin, and H. Meyer, *Helv. Chim. Acta*, **33**, 1271 (1950).
(17) J. K. Faulkner and D. Woodcock, *J. Chem. Soc.*, 1187 (1965).
(18) W. Hepworth and G. J. Stacey, British Patent 1,099,389 (1968)
(19) D. F. Jones, R. H. Moore, and G. C. Crawley, *J. Chem. Soc. C*, 1725 (1970).
(20) D. Tabone, *Bull. Soc. Chim. Biol.*, **40**, 965 (1958).
(21) W. Hepworth, British Patent 1,188,416 (1970).
(22) A. A. Smith and S. B. Wortis, *Biochim. Biophys. Acta*, **40**, 569 (1960).
(23) T. P. Dvornikova, G. K. Skryabin, and N. N. Suvorov, *Mikrobiologiya*, 37, 228 (1968); *Chem. Abstr.*, 69, 17012 (1968).
(24) W. Hepworth and T. W. Thompson, British Patent 1,121,922 (1968).
(25) J. A. Dale and H. S. Mosher, *J. Org. Chem.*, **35**, 4002 (1970).
(26) P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, 173 (1946).