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ON THE BIOSYNTHESIS OF CYCLOPIAZONIC ACID

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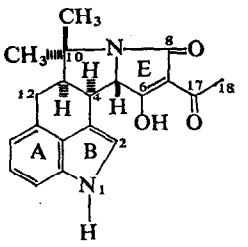
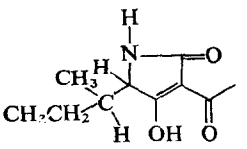
Abstract—The biosynthesis of cyclopiazonic acid (I) was studied in *Penicillium cyclopium* Westling by radiolabelling experiments. Evidence is presented that cyclopiazonic acid is derived from tryptophan, a C_5-unit formed from mevalonic acid and two molecules of acetic acid. Bissecodehydrocyclopiazonic acid (X) is a direct precursor of cyclopiazonic acid.

INTRODUCTION

Penicillium cyclopium Westling is one of the fungi which may be responsible for mycotoxicoses of farm animals.[1,2] Investigations[2,3] of toxigenic strains of this fungus showed that the major toxic compounds being produced differ from one strain to another. One of these toxic substances is cyclopiazonic acid (I)[2] which was produced in good yield by the strain



I



II

FIG. 1.

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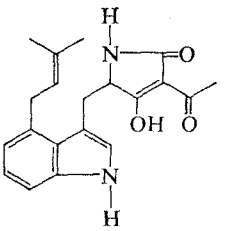
[1] J. L. ALBRIGHT, S. D. AUST, J. M. BYERS, T. E. FRITZ, B. O. BRODIE, R. E. OLSEN, R. P. LINK, J. SIMON, H. E. RHOADES and R. L. BREWER, *J. Am. Vet. Med. Assoc.* **144,** 1013 (1964).
[2] B. J. WILSON, C. H. WILSON and A. W. HAYES, *Nature* **220,** 77 (1968).
[3] C. W. HOLZAPFEL, *Tetrahedron* **24,** 2101 (1968).

C.S.I.R. 1082 of *P. cyclopium* Westling isolated from ground-nuts. Maize meal was used originally for the large-scale cultivation of this toxic strain. Subsequently, Wilkins and Holzapfel[4] found that this strain grown in stationary or shake culture on complex liquid media produced cyclopiazonic acid. With the possibility of biosynthetic studies in mind, a shake culture on a purely synthetic medium was preferred. Wilkins and Holzapfel[4] showed that a (basically) Czapek medium with sodium nitrate as nitrogen source and a trace element supplement supported a high yield of cyclopiazonic acid. The maximum rate of cyclopiazonic acid production was attained when growth, measured as the rate of change of total mycelial nitrogen, had practically ceased.

Structure analysis of cyclopiazonic acid suggested that it is probably derived either from tryptophan, a C_5-unit formed from mevalonic acid and two molecules of acetic acid or from tryptophan and two C_5-units formed from mevalonic acid. The first possibility would be analogous to the formation[5] of tenuazonic acid (II) in *Alternaria tenius* Auct. from one



X

FIG. 2.

molecule of L-isoleucine and two molecules of acetic acid. Labelled tryptophan, mevalonate and acetate were therefore used for the investigation of the biosynthesis of cyclopiazonic acid. The isotopically labelled substrates were added to the culture medium after mycelial growth had practically ceased.

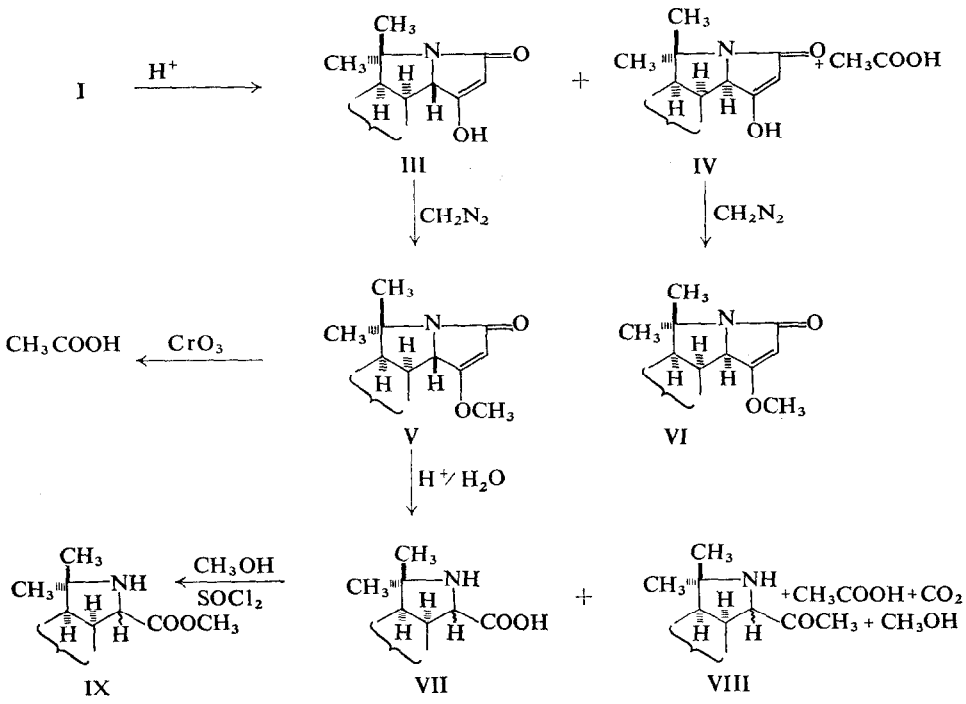
RESULTS AND DISCUSSION

For the biosynthetic studies, *Penicillium cyclopium* Westling strain 1082 was grown in shake culture on the synthetic medium described previously. The labelled substrates ([1-^{14}C]sodium acetate, [2-^{14}C]mevalonic acid and DL-tryptophan, universally ^{14}C-labelled in the benzene ring) were added on day 6 after the start of the fermentation. Cyclopiazonic acid was isolated 24 hr after the addition of the labelled substrates. The toxin was purified by chromatography and crystallized to constant activity. The following incorporations were obtained: 3·5, 7·0 and 24·7% from labelled acetic acid, DL-mevalonic acid and DL-tryptophan, respectively. The efficient incorporation of tryptophan was regarded as evidence that it is a direct precursor of cyclopiazonic acid.

Cyclopiazonic acid, labelled from [1-^{14}C]acetate and [2-^{14}C] mevalonic acid was degraded by chemical methods summarized in Scheme 1.

Cyclopiazonic acid underwent a retro-Claisen reaction when a dilute solution of the compound in 0·1 N H_2SO_4-methanol (1:1) was heated under reflux for 22 hr. One mol of

[4] D. C. WILKINS and C. W. HOLZAPFEL, unpublished work.
[5] C. E. STICKINGS and R. J. TOWNSEND, *Biochem. J.* **78,** 412 (1961).



SCHEME 1.

acetic acid (representing carbons 17 and 18) was obtained together with desacetylcyclopiazonic acid (III) and desacetylisocyclopiazonic acid (IV). These desacetyl compounds were converted into the corresponding *O*-methyl derivatives (V) and (VI) by reaction with diazomethane. Part of the acetic acid was converted into the *p*-bromophenacyl ester while the remainder was degraded by the Schmidt procedure to give CO_2, collected as barium carbonate. The acetic acid obtained from [2-^{14}C]mevalonate derived cyclopiazonic acid was essentially inactive while the specific molar activity of the acetic acid obtained from [1-^{14}C]-acetate labelled cyclopiazonic acid accounted for 35 per cent of the specific molar activity of the starting material. This labelled acetic acid carried essentially all its activity in the carboxyl group.

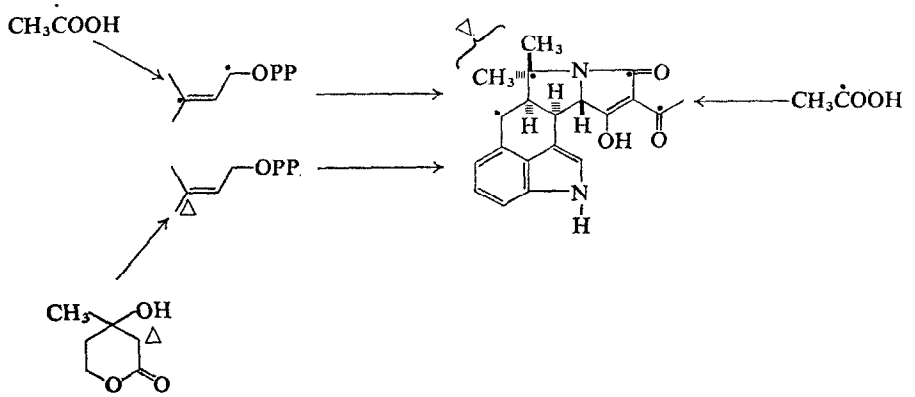
Stickings[6] found that the hydrolysis of tenuazonic acid (II) resulted in the formation of 3-amino-4-methylhexan-2-one, acetic acid and CO_2. Desacetyltenuazonic acid and desacetylisotenuazonic acid were intermediates in this reaction. It was therefore expected that *O*-methyldesacetylcyclopiazonic acid (V) would yield the corresponding aminoketone (VIII) on prolonged heating with dilute H_2SO_4. However, it was found that following hydrolysis of the methoxy-group, ring E suffered cleavage by two pathways, *viz.* (i) by hydrolysis of the amide grouping followed by decarboxylation (0·14 moles CO_2 formed) to give the aminoketone (VIII), and (ii) by a retro-Claisen reaction to give a *N*-acetylamino acid which was further hydrolyzed to acetic acid (0·74 moles), representing carbons 7 and 8, and the amino acid (VII), isolated as its methyl ester (IX). A portion of the acetic acid obtained from labelled starting material was converted into its *p*-bromophenacyl ester and

[6] C. E. STICKINGS, *Biochem. J.* **72**, 332 (1959).

the remainder degraded by the Schmidt procedure which yielded CO_2, collected as barium carbonate. The acetic acid obtained from [2-^{14}C]mevalonate derived (V) was essentially inactive while the acetic acid obtained from [1-^{14}C]acetate labelled (V) had a specific molar activity 33 per cent of the [1-^{14}C]acetate labelled cyclopiazonic acid. This labelled acetic acid carried essentially all its activity in the carboxyl group.

Kuhn-Roth oxidation of *O*-methyldesacetylcyclopiazonic acid (V) yielded 0·34 moles (average) of acetic acid representing carbon 10 and the *gem*-dimethyl-group. This acetic acid was degraded by the Schmidt procedure to CO_2, isolated as barium carbonate, and methylamine isolated as 2,4-dinitro-*N*-methylaniline. The 2,4-dinitro-*N*-methylaniline obtained in this way from [2-^{14}C]mevalonate labelled (V) accounted for 48 per cent* of the specific molar activity of [2-^{14}C]mevalonate derived cyclopiazonic acid while the 2,4-dinitro-*N*-methylalanine obtained from [1-^{14}C]acetate labelled (V) was essentially inactive. However, the CO_2 from a Schmidt degradation of the acetic acid obtained by Kuhn-Roth degradation of [1-^{14}C]acetate labelled (V) accounted for 15 per cent of the specific molar activity of [1-^{14}C]acetate labelled cyclopiazonic acid.

From these results it follows that the atoms from [1-^{14}C]acetate and [2-^{14}C]mevalonate are incorporated into cyclopiazonic acid as depicted in Scheme 2. The specific molar



SCHEME 2. INCORPORATION OF [1-^{14}C] ACETATE AND [2-^{14}C] MEVALONATE INTO CYCLOPIAZONIC ACID. ● AND △ DESIGNATE ^{14}C.

activities of the larger fragments obtained by degradation of cyclopiazonic acid are also consistent with this distribution of labelled atoms. The results of the radiolabelling experiments are summarized in Tables 1 and 2. It follows that cyclopiazonic acid is derived from tryptophan, an isoprene unit formed from mevalonic acid and two molecules of acetic acid. Agurell[7] and Plieninger *et al.*[8] showed that dimethylallyl pyrophosphate, derived from mevalonic acid, is the active isoprene unit in the biological alkylation of tryptophan on the pathway to the ergoline skeleton. It is probable that mevalonic acid is similarly incorporated into cyclopiazonic acid after conversion into γ,γ-dimethylallyl pyrophosphate.

* This value should be multiplied by 2 in order to account for the possibility that the radioactivity was distributed equally between the two methyl groups or that the radioactivity is located in only one of the methyl groups. In the latter case, Kuhn-Roth oxidation would yield an equal amount of labelled and unlabelled acetic acid.

[7] S. AGURELL, *Acta Pharm. Suecica* **3**, 71 (1966).
[8] H. PLIENINGER, H. IMMEL and A. VÖLKL, *Ann. Chem.* **706**, 223 (1967).

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN CYCLOPIAZONIC ACID PRODUCED BY *P. cyclopium* WESTL. FROM [1-^{14}C] SODIUM ACETATE

Compound	Specific molar activity μc mmoles^{-1}	Relative molar activity
Cyclopiazonic acid I	12·76	100
Acetate (C 17, 18): *p*-bromophenacylacetate	4·47	35·0
CO_2 (C 17):$BaCO_3$	4·35	34·1
O-Methyldesacetylcyclopiazonic acid (V)	8·14	63·8
Acetate (C 7, 8):*p*-bromophenacylacetate	4·21	33·0
CO_2 (C 8):$BaCO_3$	4·04	31·7
Aminoester (IX)	6·176	48·4
Acetate (*gem*-dimethyl, C 10):*p*-bromophenacylacetate	1·94	15·2
CO_2 (C 10):$BaCO_3$	1·77	13·9
CH_3NH_2 (*gem*-dimethyl):2,4-dinitro-*N*-methylalinine	0·102	0·08

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN CYCLOPIAZONIC ACID PRODUCED BY *P. cyclopium* WESTL. FROM [2-^{14}C] MEVALONIC ACID LACTONE

Compound	Specific molar activity μc mmoles^{-1}	Relative molar activity
Cyclopiazonic acid (I)	17·82	100
Acetate (C 17, 18):*p*-bromophenacylacetate	0·0053	0·03
O-Methyldesacetylcyclopiazonic acid (V)	17·99	100·8
Acetate (C 7, 8):*p*-bromophenacylacetate	0·007	0·04
Amino ester (IX)	17·53	98·4
Acetate (*gem*-dimethyl, C 10):*p*-bromophenacylacetate	8·48	47·6
CO_2 (C 10):$BaCO_3$	0·0018	0·01
CH_3NH_2 (*gem*-dimethyl):2,4-dinitro-*N*-methylaniline	8·57	48·1

It is of interest that acetic acid is incorporated more than twice as efficiently into the acetoacetate portion of cyclopiazonic acid than into the mevalonate-derived portion. This result is in agreement with the findings of Birch[9] that [1-^{14}C]acetate is incorporated to a different extent into the two acetate-derived units of fumagillin which were formed by different pathways.

Several possibilities must be considered for the order in which the simple precursors of cyclopiazonic acid are assembled. Thus, γ,γ-dimethylallyltryptophan may be an early precursor. This possibility has yet to be investigated and no attempt has been made to isolate γ,γ-dimethylallyltryptophan from *P. cyclopium* Westling. Plieninger *et al.*[8] showed that this compound is a direct precursor of the ergot alkaloids. Robbers and Floss[10] isolated this compound from a *Claviceps* strain producing mainly elymoclavine while Agurell and Lindgren[11] isolated it from a *Pennisetum* type ergot strain producing mainly elymoclavine.

During this investigation a new indole-derivative, designated bissecodehydrocyclopiazonic acid (X),[11,12] was isolated. The possibility that (X) may be a precursor of cyclopiazonic acid was suggested by the following observation. The compound (X) is already

[9] A. J. BIRCH, In *Antibiotics II: Biosynthesis* (edited by D. GOTTLIEB and P. D. SHAW), p. 152, Springer-Verlag, Berlin (1967).
[10] J. E. ROBBERS and H. G. FLOSS, *Arch. Biochem. Biophys.* **126**, 967 (1968).
[11] S. AGURELL and J. E. LINDGREN, *Tetrahedron Letters* 5127 (1968).
[12] C. W. HOLZAPFEL, R. D. HUTCHISON and D. C. WILKINS, unpublished results.

present in the mycelium at a stage when only trace amounts of cyclopiazonic acid can be detected. Initially, its concentration increases rapidly and then falls rapidly as soon as cyclopiazonic acid formation is accelerated. This behaviour could be explained if (X) is a precursor of cyclopiazonic acid, inducing the enzyme responsible for the conversion.

In order to test this proposal, attempts were made to prepare isotopically labelled bissecodehydrocyclopiazonic acid (X). It was found[4] that (X) accumulated in 7-day-old cultures of *P. cyclopium* (strain 1082), grown on a synthetic medium with a low zinc ion or ferrous ion concentration. Radioactive bissecodehydrocyclopiazonic acid was prepared by growing *P. cyclopium* on such a medium containing [1-^{14}C]sodium acetate. The labelled bissecodehydrocyclopiazonic acid (8·67 μc; 11 mg) was distributed between two flasks (100 ml medium per flask) containing 6-day-old cultures of *P. cyclopium* Westling (strain 1082) grown on the full synthetic medium. The cyclopiazonic acid isolated 48 hr later contained 67 per cent of the added label. The labelled starting material which was recovered from the fermentation accounted for 18 per cent of the added label. The result was taken as evidence that bissecodehydrocyclopiazonic acid is a direct precursor of cyclopiazonic acid. This investigation was carried further by Schabort[13] who recently described the isolation of five isoenzymes (flavoproteins) from *P. cyclopium* (strain 1082) capable of effecting the conversion of bissecodehydrocyclopiazonic acid into cyclopiazonic acid in the presence of oxygen, 2,6-dichlorophenol indophenol or cytochrome C as electron acceptor. The isoenzymes do not contain any metal ions and the addition of ferrous- and zinc-ions do not increase the reaction rate. The critical dependence of the rate of the *in vivo* conversion of (X) into cyclopiazonic acid can not, therefore, be explained in terms of their effect on the isoenzymes. However, within the cells these ions may have a profound effect on the electron carriers involved in the reaction. The mechanism of conversion of bissecodehydrocyclopiazonic acid into cyclopiazonic acid is presently being investigated.

EXPERIMENTAL

U.v. spectra were measured in MeOH and i.r. absorption in $CHCl_3$. Mass spectra were taken on a MS-9 double focusing mass spectrometer. Radioactivity was assayed on a Packard Tri-Carb Liquid Scintillation Spectrometer Model 574. Organic compounds were counted in toluene as scintillation solvent containing PPO and DM-POPOP as scintillation solute.* ^{14}C-$BaCO_3$ was assayed by suspension scintillation counting[14] in the same scintillator mixture which contained *ca.* 4%, w/v of Cab-o-sil as gelling agent. All samples were counted for a minimum of 10^4 counts and corrections were made for losses due to self-absorption. For preparative TLC, chromatoplates were coated with Merck's Silica Gel G containing a fluorescent indicator.

The Preparation of Labelled Cyclopiazonic Acid (I)

Penicillium cyclopium Westling strain 1082 from the culture collection of the Microbiological Research Group, Council for Scientific and Industrial Research, Pretoria was used in the investigation. The fungus was grown at 25° in shake culture (150 rev/min) in a synthetic medium (100 ml per 500 ml flask) with following composition: glucose (60 g), $NaNO_3$ (4·2 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), KCl (0·5 g), K_2HPO_4 (1 g), $Na_2B_4O_7 \cdot 10H_2O$ (0·7 mg), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0·5 mg), $CuSO_4 \cdot 5H_2O$ (0·3 mg), $MnSO_4 \cdot H_2O$ (0·11 mg), $ZnSO_4 \cdot 7H_2O$ (17·6 mg) and $FeSO_4 \cdot 7H_2O$ (10 mg), deionized water (1 l.): all flasks were inoculated with a standard homogenized culture inoculum prepared from the mycelium of a 2-day-old culture of *P. cyclopium* Westling grown on the above synthetic medium.

The labelled substrates were added on day 6 after the start of the fermentation. Thus, 600 μc [1-^{14}C] sodium acetate was distributed between three flasks. After 24 hr the culture was filtered, the filtrate acidified and extracted with $CHCl_3$. The mycelium was continuously extracted with $CHCl_3$. The combined $CHCl_3$ extracts were extracted with saturated aq. $NaHCO_3$. The aqueous phase was acidified with 4 N HCl and

* Packard Scintillation Grade.

[13] J. C. SCHABORT, *International Symposium on the Control of the Human Environment*, Abst, p. 62, Johannesburg (1969).

[14] H. J. CLULEY, *Analyst* **87**, 170 (1962).

extracted with $CHCl_3$. The $CHCl_3$ was evaporated and the residue chromatographed[3] on cellulose powder impregnated with $HCONH_2$–$(COOH)_2$ (50:3). Radioactive cyclopiazonic acid (203 mg) was eluted with hexane–C_6H_6 (3:1). This material was diluted with inactive cyclopiazonic acid (402 mg) and crystallized (from MeOH–$CHCl_3$) to constant radioactivity. It had m.p. 245–246° (lit.,[3] m.p. 245–246°), M^+, 336·1468 (Calc. for $C_{20}H_{20}N_2O_3$ requires M, 336·1474), specific activity (SA) 12·76 μc mmoles^{-1} (incorporation: 3·45%).

In a second radiolabelling experiment, 300 μc [2-^{14}C]DL-mevalonic acid lactone was distributed between 3 flasks. The cyclopiazonic acid isolated (129 mg) 24 hr later was diluted with inactive cyclopiazonic acid (248 mg) and crystallized to constant radioactivity. It had SA 17·82 μc mmoles^{-1} (incorporation 7·0%).

In a third radiolabelling experiment 100 μc DL tryptophan (benzene ring-^{14}C) was distributed between 2 flasks. The cyclopiazonic acid (85 mg) isolated 24 hr later was crystallized to constant radioactivity. It had SA 97·36 μc mmoles^{-1} (incorporation 24·72%).

Hydrolysis of Labelled Cyclopiazonic Acid

A solution of labelled cyclopiazonic acid (I) (250 mg) in 0·1 N H_2SO_4 aq. (250 ml) and MeOH (250 ml) was heated under reflux in N_2 for 22 hr. The mixture was cooled and neutralized with $NaHCO_3$ and exhaustively extracted with $CHCl_3$. The aqueous phase was reacidified (H_2SO_4) and steam-distilled to remove acetic acid formed in the reaction. The distillate gave a titre of 6·95 ml (average) of 0·1 N NaOH. The neutralized distillate was evaporated to dryness. A portion of the sodium acetate was converted[15] into the *p*-bromophenacylacetate and crystallized to constant radioactivity. (Found: SA 4·47 and 0·0053 μcm moles^{-1} from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively.) The remainder of the sodium acetate was subjected to a Schmidt decarboxylation[16] which released CO_2; collected as $BaCO_3$ (0·8–0·9 moles). (Found: SA 4·35 μc mmoles^{-1} from [1-^{14}C]acetate-derived material.)

The above $CHCl_3$ extract was evaporated and the residue treated with CH_2N_2 in Et_2O–MeOH (9:1). The excess of CH_2N_2 was decomposed with HOAc and the solvent evaporated. The reaction product was separated on preparative chromatoplates in $CHCl_3$–MeOH (9:1). The two main absorbing bands were eluted with MeOH. Band 1 (R_f 0·68) yielded *O*-methyldesacetylcyclopiazonic acid (V) (155 mg) which was crystallized to constant radioactivity from $CHCl_3$–Et_2O. It had m.p. 254–255° (lit.[3] 253–254°), M^+ 308·1529 (Calc. for $C_{19}H_{20}N_2O_2$: M, 308·1525). (Found: SA 7·95 and 17·99 μc mmoles^{-1} from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively).

The second band (R_f 0·58) yielded *O*-methyldesacetylisocyclopiazonic acid (VI) (71 mg), m.p. 238–239° (from $CHCl_3$), lit.[3] m.p. 236–238°. This material was not crystallized to constant radioactivity.

Hydrolysis of Labelled O-methyldesacetylcyclopiazonic Acid

A solution of labelled *O*-methyldesacetylcyclopiazonic acid (V) (150 mg) in 1 N H_2SO_4 (85 ml) and MeOH (85 ml) was heated under reflux for 50 hr. The CO_2 (0·14 moles) formed during the reaction was carried by a stream of N_2 into a solution of $Ba(OH)_2$. A portion (1/4) of the reaction mixture was steam-distilled to remove acetic acid formed in the reaction. The distillate gave a titre of 0·9 ml (average) of 0·1 N NaOH (0·74 moles). The neutralized distillate was evaporated to dryness and a portion of the sodium acetate converted into *p*-bromophenacylacetate and crystallized to constant radioactivity. (Found: SA 4·21 and 0·007 μc mmoles^{-}1 from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively). Schmidt decarboxylation of the acetic acid obtained from [1-^{14}C]acetate labelled (V) gave CO_2, collected as $BaCO_3$, which had SA 4·04 μc mmoles^{-1}.

The remainder of the reaction mixture was exhaustively extracted with $CHCl_3$. The $CHCl_3$ was evaporated and the residue separated on a preparative chromatoplate in MeOH–$CHCl_3$ (1:9). The main absorbing band (R_f 0·58) was eluted with MeOH. Evaporation of the solvent gave the *aminoketone* (VIII) (6·5 mg, average) which was crystallized from Et_2O–hexane. It had m.p. 184–185°, ν_{max} 3475, 3300 (br) and 1706 cm^{-1}, λ_{max} 223, 275 (sh), 280 and 292 mμ (log ϵ 4·50, 3·77, 3·80 and 3·65, respectively). (Found: M^+, 268·1571 $C_{17}H_{20}N_2O$ requires: M, 268·1576.) This compound was not crystallized to constant radioactivity.

The aqueous phase was evaporated *in vacuo*. The residue was dissolved in MeOH (3 ml) containing $SOCl_2$ (0·33 ml), and the solution heated under reflux for 2 hr. The solvent was removed under reduced pressure and the residue separated on a preparative chromatoplate in MeOH–$CHCl_3$ (1:9). The main absorbing band (R_f 0·48) was eluted with MeOH. Evaporation of the solvent gave the *amino ester* (IX) (25·5 mg, average) crystallized from MeOH it had m.p. 99–100°, ν_{max} 3475, 3300 (br) and 1725 cm^{-1}, λ_{max} 224, 274 (sh), 281 and 292 nm (log ϵ 4·52, 3·79, 3·81 and 3·68, respectively). (Found: M^+, 280·1519. $C_{17}H_{20}N_2O_2$ requires: M, 280·1525). This material had SA 6·176 and 17·53 μc mmoles^{-1} from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively.

[15] A. I. VOGEL, *Practical Organic Chemistry*, p. 362, Longmans, London (1961).
[16] E. F. PHARES, *Arch. Biochem. Biophys.* **33**, 173 (1951).

Kuhn-Roth Degradation of Labelled O-Methyldesacetylcyclopiazonic Acid

Compound (V) (50 mg) in 4 N chromic acid–conc. H_2SO_4 (4:1, v/v) (5 ml) was heated under reflux for 4 hr. The acetic acid was steam-distilled and titrated with 0·1 N NaOH (average yield, 0·34 moles). A portion of the NaOAc was converted into the *p*-bromophenacylacetate (SA 1·94 and 8·48 μc mmoles^{-1} from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively). The remainder of the NaOAc was degraded by the Schmidt procedure to give CO_2 and CH_3NH_2 assayed as $BaCO_3$(SA 1·77 and 0·0018 μc mmoles^{-1} from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively) and 2,4-dinitro-*N*-methylaniline (SA 0·102 and 8·57 μc mmoles^{-1}) from [1-^{14}C]acetate- and [2-^{14}C]mevalonate-derived material, respectively).

The Preparation of Labelled Bissecodehydrocyclopiazonic Acid

Penicillium cyclopium Westling strain 1082 was grown on shake culture in a medium which differed from the synthetic medium described previously in that the amount of $ZnSO_4 \cdot 7H_2O$ was reduced to $7{\cdot}04 \times 10^{-2}$ mg/l. On day 6 after the start of the fermentation, 1·3 mc [1-^{14}C]sodium acetate was distributed between 4 flasks and 24 hr later the mycelium and culture medium were extracted with $CHCl_3$. The combined $CHCl_3$ extracts were extracted with saturated aq. $NaHCO_3$. The aq. phase was acidified, extracted with $CHCl_3$ and the $CHCl_3$ evaporated. The residue (63 mg) was separated by chromatography on Whatman No. 3MM filter paper impregnated with $HCONH_2$–$(COOH)_2$ (30:1). The chromatogram was developed with hexane–C_6H_6 (3:1) and the two main absorbing bands eluted with MeOH. Band 1 (R_f 0·61) yielded cyclopiazonic acid (15 mg). Band 2 (R_f 0·47) yielded *bissecodehydrocyclopiazonic acid* (X) (34 mg) which was crystallized to constant radioactivity from $CHCl_3$–Et_2O. It had m.p. 168–169°, λ_{max} 225, 276 and 296 (sh) nm (log ϵ 4·55, 4·28 and 4·07, respectively). (Found: M^+, 338·1622 $C_{20}H_{22}N_2O_3$ requires: M, 338·1630). It had SA 266·4 μc mmoles^{-1} (incorporation: 2%).

Incorporation of Labelled Bissecodehydrocyclopiazonic Acid into Cyclopiazonic Acid

Penicillium cyclopium Westling strain 1082 was grown on shake culture in the complete synthetic medium described before. On day 6 after the start of the fermentation, a solution of [1-^{14}C]acetate-derived bissecodehydrocyclopiazonic acid (X) (11 mg, 266·4 μc mmoles^{-1}) in 0·6 ml acetone was distributed between 2 flasks. After 48 hr the mycelium and culture medium was extracted with $CHCl_3$. The aq. $NaHCO_3$ soluble fraction of this extract was separated on Whatman No. 3MM filter paper impregnated with $HCONH_2$–$(COOH)_2$ (30:1) using hexane–C_6H_6 (3:1). The band at R_f 0·61 yielded cyclopiazonic acid (86 mg). After crystallization to constant radioactivity from $CHCl_3$–MeOH it had SA 22·7 μc mmoles^{-1} (incorporation 67%). The band at R_f 0·47 yielded bissecodehydrocyclopiazonic acid (2·5 mg) which after dilution with inactive (X) (15 mg) and crystallization to constant radioactivity it had SA 29·96 μc mmoles^{-1} and this accounted for 18% of the added radioactivity.