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Biosynthesis of Cyclopiazonic Acids in *Penicillium cyclopium*: The Isolation of Dimethylallylpyrophosphate:*cyclo*-Acetoacetyltryptophanyl Dimethylallyltransferase

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During the production of β-cyclopiazonic acid (βCA) by *Penicillium cyclopium*, the following points are noted: (a) Dimethylallylpyrophosphate (DMAPP), which is incorporated into α-cyclopiazonic acid (αCA) *in vivo*, stimulates overall CA synthesis, whereas tryptophan, although incorporated into αCA, inhibits overall CA synthesis. (b) A previously suggested substrate, γ,γ-dimethylallyltryptophan, is not a precursor of αCA. (c) The accumulation of *cyclo*-acetoacetyl-L-tryptophanyl (cAATrp) is described in both the culture medium and mycelium with increasing growth. (d) A cell-free extract of mycelium will catalyze the conversion of exogenous cAATrp and exogenous DMAPP into βCA in 1:1 stoichiometry, the βCA being bound to a protein.

INTRODUCTION

In 1968 Holzapfel (*1*) isolated and characterized cyclopiazonic acid (αCA),[2] a toxin from *Penicillium cyclopium* Westling strain 1082. Later Holzapfel et al. (*2*) discovered a new metabolite, *bis*secodehydrocyclopiazonic acid (βCA), which was shown to be a direct precursor of αCA. This was later confirmed by the isolation of five isoenzymes which caused the transformation (*3*). During the conversion of β- to αCA, the final cyclization step, formation of the new carbon–carbon bond has been shown to occur from the side of the molecule opposite to that of proton removal (*4*).

After feeding tryptophan (Trp), mevalonic acid and acetate, all bearing ^{14}C, it was suggested that dimethylallyltryptophan might be an early precursor (*5*). This suggestion was in keeping with the biosynthetic route of the clavine alkaloids (*6*). In fact, the route

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[2] Abbreviations used are: Trp, tryptophan; α- and βCA, α- and β-cyclopiazonic acid; cAATrp, *cyclo*-acetoacetyl-L-tryptophanyl; DMAPP, dimethylallylpyrophosphate; IPP, isopentenyl pyrophosphate; CFE, cell-free extract.

is via the transfer of a C_5 moiety from DMAPP to α-acetyl-γ-(β-indolyl)methyltetramic acid (*cyclo*-acetoacetyl-L-tryptophanyl) (cAATrp) (*7*). This reaction is mediated by the enzyme dimethylallylpyrophosphate:cycloacetoacetyltryptophanyl dimethylallyltransferase which, throughout this paper, will be referred to as secondary transferase ("S") to distinguish it from dimethylallylpyrophosphatetransferase (EC 2.5.1.1.), primary transferase ("T").

This isoprenylation of Trp or a derivative is not novel; in 1972 Allen (*8*) reported the transfer of the dimethylallyl group to cycloalanyltryptophanyl in position 2 of the indole nucleus in *Aspergillus amstelodami*, while in 1971 the enzyme responsible for 4-γγ-dimethylallyltryptophan itself was isolated from *Claviceps* sp. (*6*), thus reinforcing considerable evidence of this route in the clavine alkaloids.

MATERIALS

Merck silica gel F_{254} tlc plates, precoated 0.25-mm thick, were obtained from Merck, Germany. [1-^{14}C]Isopentenylpyrophosphate, L-[G-^{3}H]tryptophan, L-[*methylene*-^{14}C]tryptophan, L-[*carboxyl*-^{14}C]tryptophan and [2-^{14}C]mevalonic acid were all obtained from Amersham, England.

SYNTHESES

Dimethylallylpyrophosphate (DMAPP) and Isopentenylpyrophosphate (IPP)

These were prepared by the general method described by Cornforth and Popják (*9*) and purified by chromatography on Sephadex G-25 instead of silica gel, which had the advantage of increased loads and permanency and yielded a purer product. The column was 80 × 2.6 cm, equilibrated and eluted with propanol:concentrated NH_4OH (65:35). The eluate was monitored using silica TLC plates developed by propanol:concd NH_4OH:0.001 *M* EDTA (6:2:2), and the phosphates detected with a molybdate spray (*10*). R_f's of the monophosphates and pyrophosphates of the two alcohols were 0.33–0.44 and 0.18–0.24, respectively. Analysis of the pyrophosphates indicated a purity of 95%.

[4,5-^{14}C]Dimethylallylalcohol, [4,5-^{14}C]Dimethylallylmonophosphate and [4,5-^{14}C]-Dimethylallylpyrophosphate

Dimethylallylalcohol was prepared by the method of Plieninger and Immel (*11*) and phosphorylated according to Cornforth and Popják (*9*).

[1-^{14}C]Dimethylallylpyrophosphate

It was possible to produce [1-^{14}C]DMAPP with relatively high specific activities (0.1–60.0 μCi/μmole) from commercial [1-^{14}C]IPP by using isopentenyl pyrophosphate isomerase (EC 5.3.3.2) obtained from pig liver (*12*). The conversion incubation was: [1-^{14}C]isopentenyl pyrophosphate, 100 nmoles, 6 μCi; Tris–acid maleate, pH 6.0,

50 μmoles; $MnSO_4 \cdot 7H_2O$, 10 μmoles; isomerase, 2.5 mU (1 mU will produce 1 nmole of DMAPP in 1 min at 30°C); and H_2O to 1.0 ml. The isomerization was carried out at 30°C for 30 min and stopped by immersion in boiling water for 3 min. After cooling, 10 μmole of DMAPP was added to the solution and the denatured protein removed by centrifugation. Addition of a few drops of concd NH_4OH to the supernatant precipitated $Mn(OH)_2$ after 16 hr. The mixture of isomers could be separated chromatographically (*13*). The two isomers were detected by scanning for ^{14}C, CMAPP had an R_f of 0.35–0.40 and IPP, 0.50–0.55. Integration of the peaks gave a ratio DMAPP: IPP of 2:1 in several experiments. This was confirmed by acidifying a sample (hydrolyzing DMAPP to methylvinylcarbinol) saturating with $MgSO_4 \cdot 7H_2O$ and extracting with toluene several times. This figure is lower than those reported by Holloway and Popják (*12*) and Shah et al. (*13*) who quote K_{eq} of 5 and 6.7, respectively.

The DMAPP was eluted from the paper using 0.1 *M* NH_4OH and taken to dryness. Sufficient Tris–acid maleate (pH 8.0, 1 m*M*) was added to give a DMAPP concentration of 10 μmoles/ml.

*α-Acetyl-γ-(β-indolyl)-methyltetramic acid (cyclo-acetoacetyl-*L*-tryptophanyl)*

Five hundred milligrams of [G-^{3}H]Trp, 408 μCi/mmole was treated with 0.38 ml of thionyl chloride in 5.0 ml of methanol. The solution was kept at 0°C for 16 hr and 20°C for 1 hr before removing the reagents with a stream of dry N_2. The dried residue, dissolved in 5 ml of methanol, was treated at room temperature for 15 min with sodium methoxide (60 mg Na in 0.8 ml methanol). The mixture was cooled to −10°C, treated with 0.3 ml of diketene and stirred at 20°C for 20 hr, then filtered and concentrated. The concentrate was separated on silica gel to give *N*-acetoacetyltryptophan methyl ester (604 mg), 550 mg of which was dissolved in 15 ml of benzene, treated with sodium methoxide (40 mg Na/5 ml methanol) and refluxed for 50 min. The mixture was poured on ice and extracted with benzene. On acidification, the aqueous phase was extracted into chloroform to give α-acetyl-γ-(β-indolyl)-methyltetramic acid, generally ^{3}H labeled in Trp (450 mg), mp 167–168°C (from benzene). *Anal.* Found: C, 63.85; H, 6.20; N, 15.70. $C_{15}H_{14}N_2O_3$ requires: C, 64.14; H, 6.01; N, 15.57%.

γγ-4-Dimethylallyl-[G-^{3}H]tryptophan. We thank Professor C. W. Holzapfel of the Randse Afrikaanse University, Johannesburg, for a generous gift of this compound.

METHODS

Protein Determination

Protein was determined by the method of McGrath (*14*), modified to contain half the reported concentration of cyanide.

Microbiological

P. cyclopium Westling strain 1082 was grown at 25°C in shake culture (200 rpm) in a synthetic medium (*4*).

Radioactive Feeding and Determination

The time and duration of feeding depended on the specific activity of the precursor. If this was low, then a pool of the labeled precursor was desirable over a long period of growth. Results then emphasize dilution of specific activity (i.e., specific activity of precursor/specific activity of metabolite). If specific activity was high and the concentration low, then a shorter feeding time, just past the maximum rate of secondary metabolism, was desirable, and the emphasis placed on total incorporation. The precursors were fed as aqueous solutions sterilized by filtration.

Radioactivity was determined using a Packard Tri Carb spectrometer with Triton X-100:toluene (1:2) liquid scintillation mixture (*15*).

In the case of double-labeling experiments, the isotopes were separated and estimated using a Packard sample oxidizer (Model 306).

Paper and silica chromatograms were scanned using a Berthold scanner (LB 2722).

Isolation and Estimation of α- and β-Cyclopiazonic Acids

These metabolites were isolated from the acidified, homogenized mycelium and filtrate mixture by extraction with chloroform. This was extracted with 0.1 *M* ammonia which, in turn, was acidified and reextracted into chloroform. The residue was made up to a standard volume containing about 20 mg of the cyclopiazonic acids per ml of chloroform (estimated by uv, log $\varepsilon = 4.33$ at 280 nm) and 1 ml applied to a silica gel tlc plate (20 × 20 cm) which was developed using ethyl acetate:methanol:concd NH_4OH (85:15:10). The metabolites were located by uv (αCA and βCA, $R_f = 0.53$ and 0.40, respectively) as very narrow absorbing bands then scraped off and eluted with methanol.

The methanolic solution of α- or βCA from the above tlc partition was applied to a silica gel tlc plate which had been dipped in an 8% (w/v) methanolic solution of oxalic acid and air dried. The plate was developed using chloroform:methylisobutylketone (4:1) (*16*). The αCA (R_f, 0.7) and βCA (R_f, 0.5) were detected by uv, scraped off, and eluted with chloroform, to be estimated spectrometrically and radiometrically.

Cell Breakage and Cell-free Extract (CFE)

The mycelium was washed with buffer (Tris–chloride, pH 7.9, 10 m*M*; mercaptoethanol, 2 m*M*; sodium diethyldithiocarbamate, 2 m*M*), packed into a press, frozen in Dry Ice and broken by ice shear (*17*). The broken mycelium was extracted with four volumes of the buffer by stirring for 15 min at 5°C. The CFE was obtained by centrifuging at 48 000 *g* for 1 hr at 2°C, followed by careful separation of lipids by decantation and filtration through a large-diameter filter paper.

Cell-free Biosynthesis of βCA

A 20-ml reaction mixture containing: L-[G-^{3}H]Trp, 4 μmoles, 1 μCi; [1-^{14}C]-DMAPP, 2.5 μmoles, 0.75 μCi; Tris–chloride, 1 mmole, pH 7.9; CFE, 10 ml, was incubated at 30°C for 1 hr. It was stopped by immersion in boiling water for 3 min and the denatured protein centrifuged off.

To the supernatant was added sufficient Sephadex G-25 (fine) so that an immobile

mass was formed. This was mobilized by the addition of a minimal amount of propanol: 0.1 *M* NH_4OH (70:30) and loaded onto a Sephadex G-25 (fine) partition column equilibrated with the above propanol solution.

The denatured protein was processed as shown in Scheme 1 (Fig. 1).

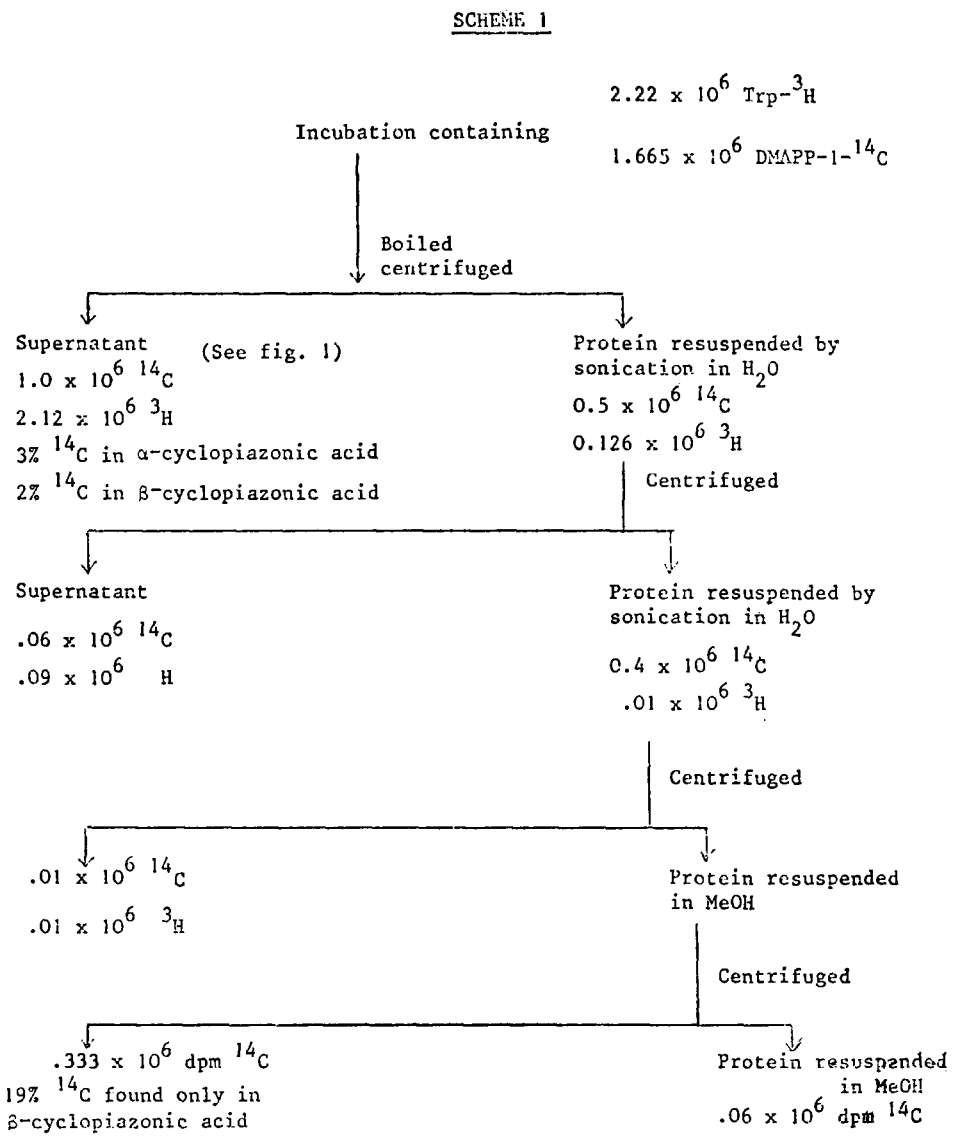

FIG. 1. Treatment of denatured protein from cell-free biosynthesis of βCA. Figures are disintegration per minute for the two isotopes.

Assay of Secondary Dimethylallyltransferase ("S")

The incubation consisted of: Tris–acid maleate, 50 μmoles, pH 7.2; [1-^{14}C]DMAPP, 100 mmoles, 80 000 dpm; cAATrp, 100 nmoles, and 1–5 mUnits of enzyme in a total

TABLE 1

RADIOACTIVE PRECURSOR INCORPORATION INTO α-CYCLOPIAZONIC ACID

Expt. No.[a]	Compound	Feeding (day-on-day-off)	Total radioactivity (10^{-6} dpm)	Specific activity (mμCi/μmole)	Specific activity of cyclopiazonic acid (mμCi/μmole)	Dilution[b] of specific activity	Yield (μmoles per 300 ml)	Incorporation (%)
1	L-tryptophan, *methylene*-^{14}C-labeled	2–5	8.44	12.6	1.25	10	357	12
2	DL-tryptophan, *methylene*-^{14}C-labeled	2–5	13.10	9.7	2.78	3.5	357	17
3	No addition	—	—	—	—	—	946	—
4	Dimethylallyl pyrophosphate, 4,5-^{14}C-labeled	2–5	2.90	4.0	0.17	23	1351	17.6
5	Dimethylallyl monophosphate, 4,5-^{14}C-labeled	2–5	6.90	4.0	0.03	123	893	0.9
6	Dimethylallyl alcohol, 4,5-^{14}C-labeled	2–5	6.45	4.0	0.02	200	952	0.7
7	Mevalonic acid, 2-^{14}C-labeled	2–5	16.80	18.6	0.83	22	946	10.0
8	γ,γ-Dimethylallyl tryptophan (DL-[G-^{3}H]Trp)	4–5	2.02	234.0	0.028	8300	164	0.5
9	γ,γ-Dimethylallyl tryptophan (DL-[G-^{3}H]Trp)	4–5	2.02	234.0	No. ^{3}H	—	164	—
	Tryptophan, *methylene*-^{14}C-labeled		22.20	54 500	5.2	10 480		8.4
10	Tryptophan, *methylene*-^{14}C-labeled	4–5	22.20	2000	5.9	338	208	12.2

[a] Experiments 1–7 used one inoculum and identical conditions producing 1.3 ± 0.1 g mycelium/flask; three flasks per experiment each containing 100 ml of medium. Experiments 8–10 used one inoculum different from previous group producing the same dry weight of mycelium.

[b] Dilution of specific activity = specific activity of precursor/specific activity αCA.

of 1.0 ml. A blank reaction was always included where the enzyme was replaced by H_2O. The incubation was for 10 min at 30°C and was stopped by the addition of 2 drops of concd HCl. It was then extracted with benzene, 1.0 ml containing 10 μg of βCA. A 100-μl aliquot of the benzene layer was applied to a tlc silica plate and developed using ethylacetate:methanol:concd NH_4OH (85:15:10). The βCA, $R_f = 0.40$, was scraped directly into a scintillation vial and counted for 10 min after cooling for at least 2 hr.

Amino Acid Pools

These were measured on cultures grown from one inoculum. Cultures were grown as described and harvested at 12-hr intervals (10 flasks were grown per time interval). Crude extracts (uncentrifuged) were prepared as described for CFE and aliquots taken to estimate the amino acid pools.

The aliquot was boiled under reflux while stirring with chloroform:methanol (3:1). The solvents were removed by centrifugation, and the residue extracted twice with water and the supernatants mixed. The pH was adjusted to 2.0 with sulfosalicyclic acid and centrifuged. The clear aqueous material was adjusted to pH 7.0 before being taken to dryness. The residue was dissolved in 50 ml of lithium citrate buffer pH 2.2, before loading an aliquot onto a Technicon ion-exchange column (Chromo-beads, Type B) 0.6 × 140 cm. Elution was performed using a gradient in both pH and counter ion (Li^+) running from pH 3.0 to 6.8. The temperature was kept at 37°C for 5 hr and then at 71.5°C for 16 hr (Technicon Instruction Manual AAA-1).

RESULTS AND DISCUSSION

Radioactive Precursor Experiments

The production of αCA is apparently increased by addition of DMAPP and decreased by Trp (Table 1), a fact often noted during our work. In experiments 1 and 10 it seems that less Trp brings about a greater inhibition; however this anomalous result is due to a variation in the ratios of α- and βCA which can occur from inoculum to inoculum. Tables 2 and 3 confirm the effect of DMAPP and Trp on the total α- and βCA production, respectively.

TABLE 2

THE EFFECT OF DMAPP[a] ON THE PRODUCTION[b] OF α- AND βCA (TOTAL)

Culture age (days)	CA (μmoles/300 ml)	Mycelial weight (g/300 ml)	CA (μmoles/g mycelium)
2	286 (214)	3.3 (3.2)	84 (66)
3	678 (595)	3.9 (3.6)	174 (161)
4	1250 (893)	4.2 (3.6)	298 (241)
5	1339 (893)	4.3 (3.9)	311 (228)

[a] 1.0 m*M* added to medium before inoculation.

[b] Values in parentheses are controls, i.e., without exogenous DMAPP.

TABLE 3

THE EFFECT OF TRP[a] ON THE PRODUCTION[b] OF α- AND βCA (TOTAL)

Culture age (days)	α- and βCA (μmoles/300 ml)	Mycelial weight (g/300 ml)
1	36 (36)	0.6 (1.2)
2	214 (223)	2.4 (3.0)
3	381 (804)	2.7 (3.4)
4	402 (1250)	3.1 (3.7)
5	625 (1340)	3.6 (4.4)

[a] 5.0 m*M* Trp added to the media (3 × 100-ml) before inoculation.

[b] Values in parentheses are controls, i.e. without exogenous Trp.

DMAPP is also a better precursor or intermediate than either the parent alcohol or the monophosphate. In fact, what incorporation there is in the latter two cases, probably occurs via other routes because they were fed over a long time. The other suspected intermediate, dimethylallyltryptophan (*5*), was not incorporated at all when mixed with Trp, though when fed alone it exhibited 0.5% incorporation which was probably due to its catabolism. The dependence of "Dilution of specific activity" on the concentration is well illustrated in experiments 1, 2, 9 and 10. It is obvious in 9 and 10 that Trp is highly diluted by the endogenous system.

Because of the failure of dimethylallyltryptophan to be incorporated and also because Trp inhibited αCA synthesis, it was felt that a catabolite of Trp could be an intermediate. However, Table 4 indicated that all the C atoms of Trp were incorporated directly into αCA.

TABLE 4

TRYPTOPHAN INCORPORATION INTO α-CYCLOPIAZONIC ACID

Compound	Feeding (day-on–day-off)	Radioactivity (μCi)	$^{3}H/^{14}C$ in cyclopiazonic acid (dpm × 10^{-5})	Ratio
L-[G-^{3}H]tryptophan		222.0	11.73/1.2	9.8
L-tryptophan, *methylene*-^{14}C-labeled	3–4	22.2		
L-[G-^{3}H]tryptophan		222.0	10.44/0.94	11.0
L-tryptophan, *carbonyl*-^{14}C-labeled	3–4	22.2		

Amino Acid Pools

Because Trp is such an important precursor, its endogenous pools were measured over the period of production of αCa. Table 5 indicates that it exists, if at all, as an

TABLE 5

AMINO ACIDS

Age (hr)	36	48	60	72	84
Dry weight (g)[a]	3.6	6.3	8.7	9.7	11.8
Soluble protein (mg)[a]	424	719	870	408	490
α- and βCA (mmoles)[a]	0.15	0.30	0.60	1.31	1.37
Amino acid	Micromoles per gram dry weight of mycelium				
Cysteic acid	1.1	1.45	1.45	1.40	2.5
Aspartic acid	5.1	3.3	2.1	1.8	2.0
Threonine	2.9	3.3	2.0	2.3	3.8
Serine	4.0	5.8	3.2	4.7	3.8
Asparagine	0	0	0	0	0
Glutamic acid	20.0	18.2	11.8	7.7	2.7
Glutamine	39.4	24.6	13.6	17.2	13.5
Proline	1.8	2.9	1.9	1.6	2.2
Glycine	3.3	3.6	2.0	2.4	2.2
Alanine	48.8	24.6	7.6	9.8	10.6
Cysteine	0	0	0	0	0
Valine	6.5	10.5	6.3	2.3	5.7
Methionine	1.1	1.8	0.5	0.4	0.7
Isoleucine	1.5	2.9	1.4	1.6	1.8
Leucine	3.6	1.7	4.1	3.9	4.6
Tyrosine	1.5	2.2	1.3	1.4	1.8
Phenylalanine	1.8	3.3	2.1	2.2	2.5
β-Alanine	0	0	0	0	0
γ-Aminobutyric acid	25.4	52.2	18.0	22.7	27.0
Tryptophan	0	0	0	0	0
Ornithine	1.5	1.8	0.7	1.2	1.2
Lysine	3.3	4.8	3.7	3.2	2.9
Histidine	1.1	1.5	1.5	1.2	1.6

[a] Per liter of culture.

extremely small pool compared to the other amino acids. The other main feature of this table is the preponderance of glutamic acid, glutamine, alanine, and γ-aminobutyric acid which, together, make up more than 50% of the total amino acids at any time.

Cell-free Biosynthesis of βCA

The outstanding incorporation of both Trp and DMAPP into αCA in whole culture made it imperative to test the CFE for similar activity. Figure 2 shows the distribution of the two isotopes in the supernatant. About 10% of the total ^{14}C fed is shown in Fig. 2; 30% was bound to a protein which precipitated on boiling (Fig. 1) and 55% was eluted as DMAPP around tube 200 which is not shown in Fig. 2.

Tubes 23–45 when taken to dryness lost 90% of the ^{14}C. Because of its volatility, it was thought to be an alcohol derived from DMAPP. The remaining isotopes of tubes 23–45 were dissolved in methanol and the cyclopiazonates isolated by tlc as described

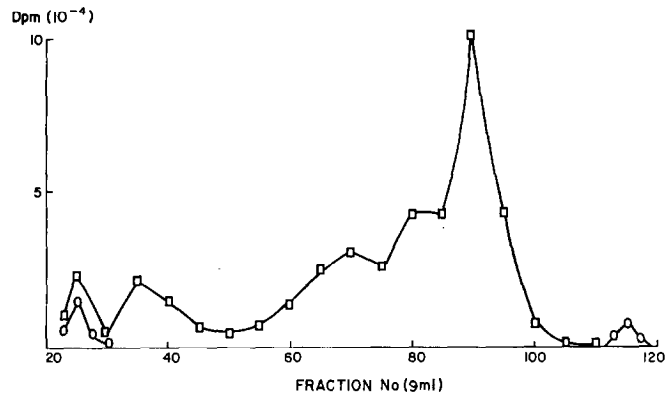

FIG. 2. Partition of supernatant from cell-free biosynthesis of βCA after heat denaturation. Eluant was propanol: 0.1 M NH_4OH (7:3) on a column (100 × 2.5 cm) of Sephadex G-25 (fine). 9-ml fractions were collected and ^{14}C(o) and ^{3}H(□) measured. [1-^{14}C]DMAPP is not shown, eluting around tube 200.

in Methods. No ^{3}H was found in either α- or βCA, but the former possessed 0.15% and the latter 0.1% of the original ^{14}C.

The denatured protein was found to bind 30% of the total ^{14}C present in the CFE incubation, 19% (still of the total ^{14}C) of which was shown to be incorporated only into βCA; there was no ^{3}H found in βCA while αCA was devoid of any radioactivity (Fig. 1).

The βCA separated from the denatured proteins (Fig. 1) had 5 mg authentic βCA added to it and was isolated using in sequence the tlc systems described (Methods), thus confirming the product as βCA.

It was clear that the CFE contained both an enzyme and a substrate which, along with DMAPP, would produce βCA. This co-substrate was, therefore, a derivative of Trp, which we showed to be α-acetyl-γ-(β-indolyl)methyl tetramic acid (*7*). An incubation similar to that described under *Assay of secondary dimethylallyltransferase* ("*S*") using [G-^{3}H]cAATrp as well as [1-^{14}C]DMAPP, yielded βCA with a ^{3}H : ^{14}C, ratio which indicated a 1:1 reaction of the substrates (Table 6). This is in keeping with the pathway shown in (Fig. 3) Scheme 2.

TABLE 6

INCUBATION OF [G-^{3}H]cAATrp (100 nMOLES, 32 000 DPM) AND [1-^{14}C]-DMAPP (100 nMOLES, 80 000 DPM) WITH 1 ML OF CELL-FREE EXTRACT[a]

	Radioactivity (dpm)		Substrate in product (nmoles)	
Product	^{3}H	^{14}C	cAATrp	DMAPP
βCA	781	1845	2.44	2.30

[a] See enzyme assay in Methods for details of incubation.

The route from Trp to cAATrp is inoperative in the CFE system described, otherwise [G-^{3}H]Trp would have appeared in βCA. Trp was readily metabolized into two new more nonpolar substances (fractions 70 and 80, Fig. 2), both of which total more than

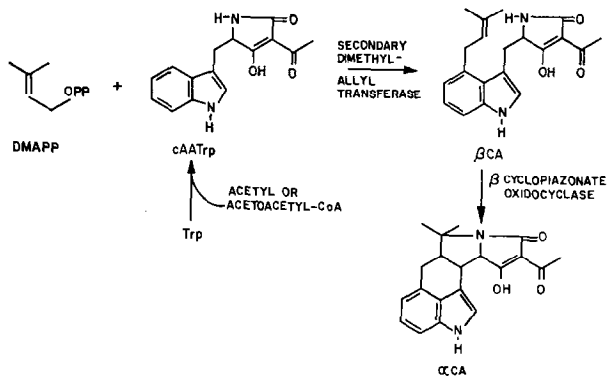

FIG. 3. The pathway of cyclopiazonic acid biosynthesis.

the remaining Trp. In view of Gatenbeck and Sierankiewiczs' work (*19*) on tenuazonic acid, another tetramic acid, it is possible that these two metabolites might be *N*-acetyltryptophan and *N*-acetoacetyltryptophan.

Variations of cAATrp with Age

cAATrp is present maximally on day 4 and is found predominantly in the culture filtrate (Table 7). However, the CFE represents a 1 to 4 dilution of the mycelium and so the concentration of the metabolite in the filtrate and the mycelium is approximately equal, which suggests a process of diffusion rather than active excretion. It perhaps represents an attempt by the cell to excrete Trp.

TABLE 7

DISTRIBUTION AND POOL VARIATION OF cAATrp WITH TIME

	Age of culture							
	2 days		3 days		4 days		5 days	
Boiled CFE (μl)	200	400	200	400	200	400	200	400
^{14}C in β-cyclopiazonic acid (dpm)	300	580	600	1170	1200	1900	1050	1500
Boiled culture filtrate (μl)	100	200	100	200	100	200	100	200
^{14}C in β-cyclopiazonic acid	100	200	1500	2200	2000	3000	1600	2750

Incubation and assay are described in Methods, cAATrp being replaced by boiled CFE or culture filtrate.

Secondary Dimethylallyltransferase

This enzyme is found only in the mycelium. It shows a direct relationship between enzyme concentration and βCA production (Table 8), over a period of 10 min (Table 9) with an excess of both substrates. The pH profile is broad, with an optimum lying between 6.9 and 7.5 (Table 10).

TABLE 8

DEPENDENCE OF β-CYCLOPIAZONIC ACID PRODUCTION ON PROTEIN CONCENTRATION[a]

Protein (μg) (3-day cell-free extract)	0	20	40	80	160	240
^{14}C in β-cyclopiazonic acid (dpm)	0	440	810	1380	2230	3030

[a] Incubation and assay are described under Methods.

TABLE 9

TIME DEPENDENCE OF β-CYCLOPIAZONIC ACID PRODUCTION[a]

Time (min)	0	3	6	10	20	30	40
^{14}C in cyclopiazonic acid (dpm)	90	634	1140	1670	2070	2170	2070

[a] Incubation is described in Methods.

TABLE 10

pH[a] DEPENDENCE OF THE SECONDARY TRANSFERASE

pH	5.4	5.9	6.5	6.9	7.5	7.9	8.5
^{14}C in β-cyclopiazonic acid (dpm)	450	1810	2220	2680	2570	2480	238

[a] Buffer: Tris–acid maleate at 0.05 *I* throughout pH range. See Methods for assay.

Presumably this secondary transferase is in direct competition with prenyltransferase (EC 5.3.3.2) for the common substrate DMAPP. The branch point thus formed will control the routing of DMAPP into polyisoprenoids (sterols, ubiquinones etc.) or secondary metabolites, providing that the enzymes are in excess and available to the same pool(s) of substrates.

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