RECENT BIOSYNTHETIC STUDIES ON ANTIBIOTICS[1]

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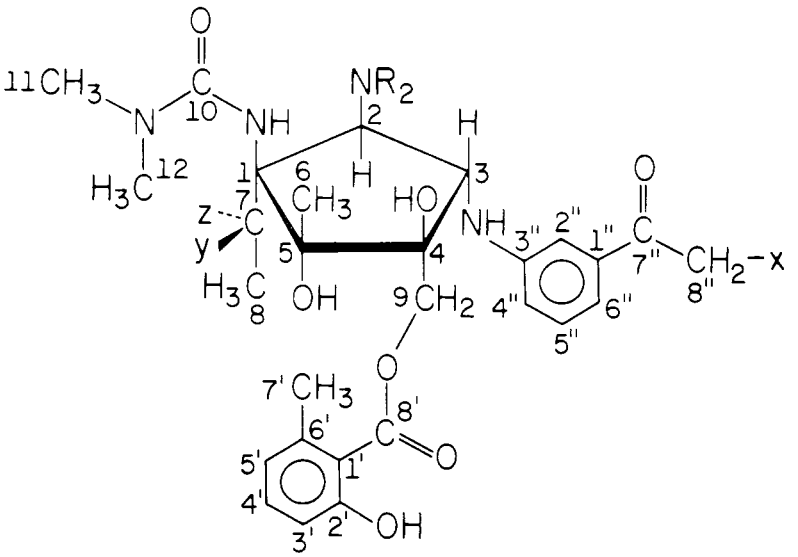
During recent years our research group has been engaged in biosynthetic studies dealing with antibiotics of two different sorts. In a continuing study we have investigated the biosynthetic origins of the very important aminocyclitol antibiotics, which include gentamicin, neomycin, streptomycin, spectinomycin, etc. This topic has been reviewed extensively within the past year (1, 2, 3).



1: X = Y = R = H, Z = OH

7: X = Y = H, Z = OH, R_2 = $=C(CH_3)_2$

8: X = Z = OH, Y = R = H

9: X = Y = Z = R = H

10: X = Z = R = H, Y = OH

Concurrently, we have studied the biosynthetic origins of a number of antibiotics more of interest for their novel structural features than for their importance as clinically useful entities. It is a portion of the latter studies that will be described here. For each of the three antibiotics to be discussed—pactamycin, bernina-

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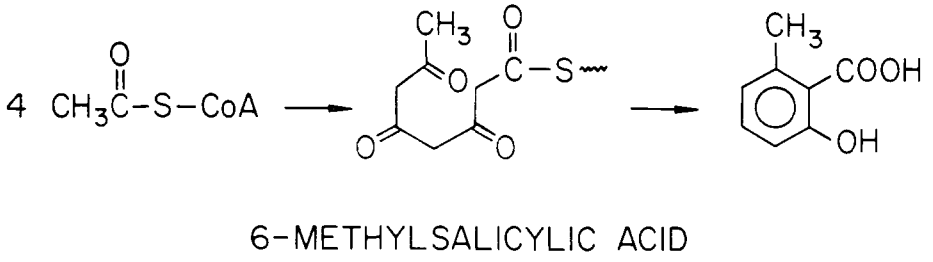
mycin, streptolydigin—two or more distinctly different biosynthetic routes appeared reasonable.

PACTAMYCIN.—The first of the structurally intriguing antibiotics, pactamycin (4, 5, 6), has potent antimicrobial activity against gram-positive bacteria (MIC 0.8 μg/ml vs. *Bacillus subtilis*), but its toxicity (LD_{50} 10.7 mg/kg, oral, in mice) precludes its use as a therapeutic agent. It is, however, of greater interest for its cytotoxicity (0.003 μg/ml vs. KB cells) and its antitumor activity (30% inhibition of Rous sarcoma virus at 0.5 mg/kg) (7). More interesting still is the structure of pactamycin (**1**), which is unique among known antibiotics. In consequence of this unique structure, we have commenced our biosynthetic investigations on the compound (8).

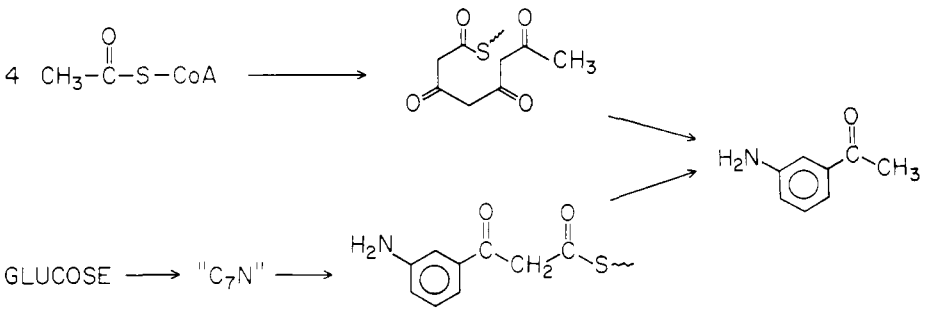
An initial inspection of the structure of the antibiotic indicates that one of the three cyclic units, the 6-methylsalicylate, almost surely must be derived from four moles of acetate (fig. 1), since the biosynthetic origins of 6-methylsalicylic acid itself are well known (9).



6-METHYLSALICYLIC ACID

FIG. 1. Biosynthetic pathway to 6-methylsalicylate.

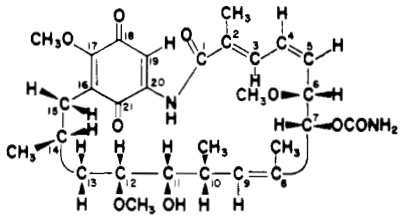
The second structural entity, the *m*-aminoacetophenone unit, could, in principle, be derived from at least two plausible biosynthetic sources (fig. 2). As one possibility, *m*-aminoacetophenone could arise, like 6-methylsalicylic acid or acetylphloroglucinol (9), from four moles of acetate. On the other hand, recent studies with other antibiotics have indicated that an aromatic "C_7N" unit in which the amino group and a carboxyl-derived carbon are in the *meta* position with respect to one another can be derived from a shikimate-related intermediate and, ultimately, from glucose *via* 3-deoxy-D-arabinoheptulosonic acid 7-phosphate. This "C_7N" unit (10) is found in a simple form in the benzoquinones geldanamycin (**2**) (11) and mitomycin (**3**) (12) and in a more complex



m-AMINOACETOPHENONE

FIG. 2. Alternative biosynthetic pathways to *m*-aminoacetophenone.

form in the naphthoquinonoid ansamycins streptovaricin D (4) (10) and rifamycin S (5) (13). The remaining methyl carbon of the C_8 unit of pactamycin could come from a variety of potential sources.



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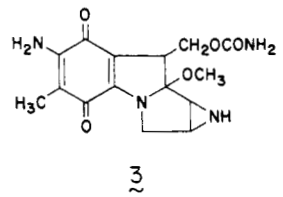

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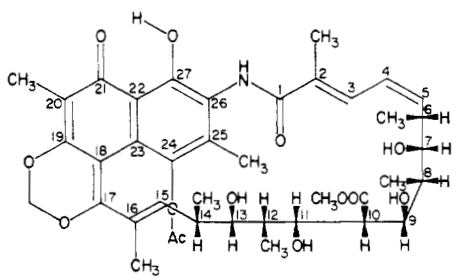

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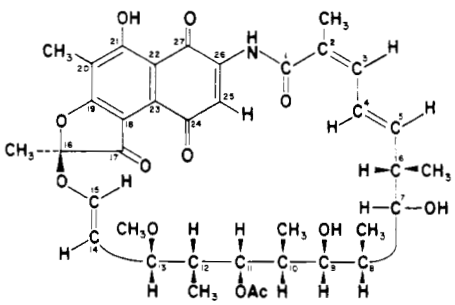

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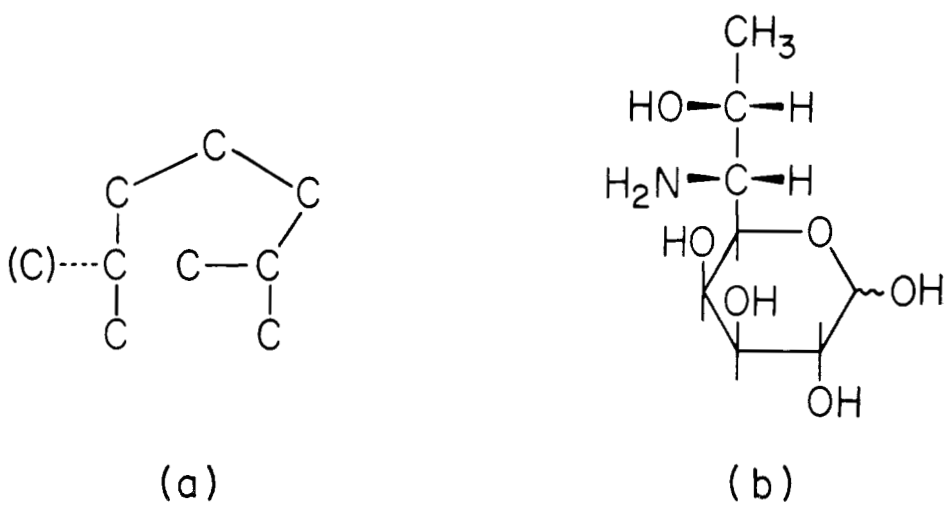
The branched, highly substituted cyclopentane ring's origins are considerably more obscure, since this is the unique unit in pactamycin. Among other routes to the cyclopentane unit, cyclization of a monoterpenoid, with loss of one carbon atom, is possible (fig. 3a), or its continuous chain of eight carbons could be related



(a) (b)

FIG. 3. a) Hypothetical construction of carbon skeleton of cyclopentane unit of pactamycin from a monoterpene. b) Lincosamine, a C_8 amino sugar hypothetically related to the same unit.

to that found in the lincosamine portion (fig. 3b) of lincomycin, which has been reported to arise from glucose and pyruvate (14). Still other possibilities are that the cyclopentane unit could be derived, like prostaglandins, *via* cyclization of a diene, or from polyketide intermediates, with rearrangement.

As with most biosynthetic investigations, the first studies were designed to ascertain appropriate precursors by establishing which ^{14}C-labeled compounds were incorporated into pactamycin. These showed (table 1) that the three

TABLE 1. Precursors incorporated.

Precursor	Pactamyçate	
	% Incorporation	Dilution
[*methyl*-^{14}C]Methionine	0.020[a]	0.54[a]
Sodium [*carboxy*-^{14}C]acetate	0.093	1.89
D-[*6*-^{14}C]Glucose	0.061	0.60

[a]Pactamycin; 60.3% of this activity recovered in pactamyçate.

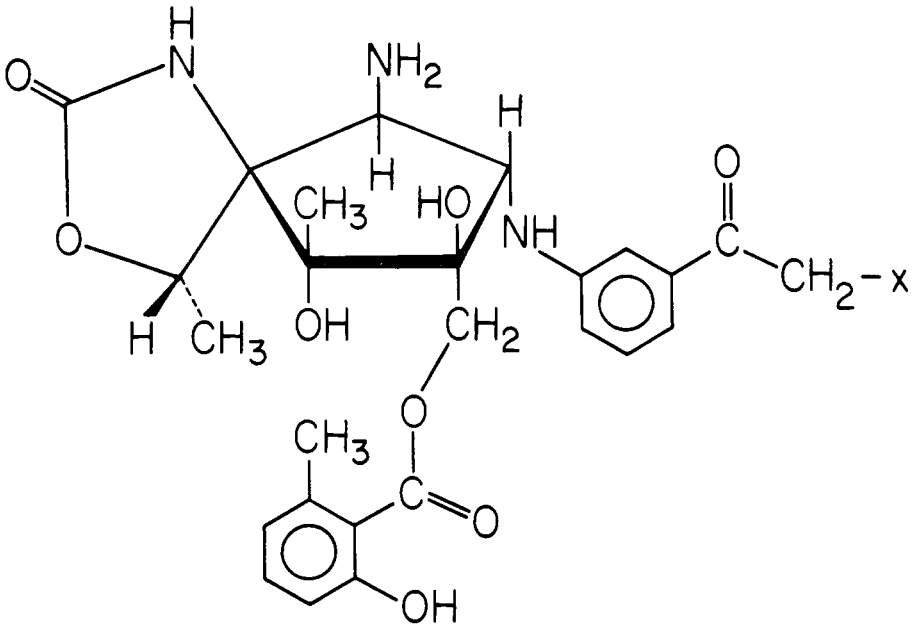
precursors acetic acid, glucose, and methionine are well incorporated; other potential precursors were much less so. The next step involved incorporation of ^{13}C-labeled precursors. Before this study was undertaken, the ^{13}C spectrum of pactamycin was first assigned (table 2) (15), based on off-resonance studies which divided the carbon atoms into methyl, methylene, methine, and quaternary carbons, together with assignments from first principles (oxygen-substituted carbon atoms at lower field than nitrogen-substituted at lower field than carbon-substituted). In addition, studies of models were carried out, which involved

TABLE 2. Chemical shifts of pactamyçate carbons.

Pactamyçate carbon	Chemical shift[a]	Pactamyçate carbon	Chemical shift[a]
1	69.8	4'	130.2
2	57.6	5'	120.2
3	70.2	6'	136.9
4	80.3	7'	19.5
5	82.0	8'	167.9
6	17.3	1"	137.2
7	75.7	2"	111.4
8	16.3	3"	149.9
9	67.2	4"	116.6
10	158.1	5"	128.6
1'	119.9	6"	115.6
2'	154.8	7"	198.4
3'	113.3	8"	26.6

[a]Parts per million from Me_4Si; Me_2SO-d_6 solutions.

m-aminoacetophenone, ethyl 6-methylsalicylate, and compounds closely related to pactamycin—pactamyçate (**6**), which lacks the dimethylamino group, and the isopropylidene adduct (**7**) of pactamycin. In both, the substituted carbons are shifted *vis-a-vis* the native antibiotic.



6 : x = H

11 : x = OH

The ^{13}C labeling studies summarized in table 3 indicate clearly that the 6-methylsalicylate portion of pactamycin is derived from acetate, with C-1', C-3', C-5' and C-7' labeled by the methyl carbon of acetate and C-2', C-4', C-6' and C-8' by the carboxyl carbon. In addition, [6-^{13}C]glucose labeled the same carbons labeled by [*methyl*-^{13}C]acetate, presumably by conversion of glucose to acetate *via* pyruvate (16).

The second unit of pactamycin, *m*-aminoacetophenone, is derived in part from acetate, but only C-8" is labeled—by the methyl carbon of acetate (table 4);

TABLE 3. Enrichment from labeled precursors.

Carbon	L-Met	Sodium acetate		D-Glucose	
	[CH_3-^{13}C]	[1-^{13}C]	[2-^{13}C]	[6-^{13}C]	[1-^{13}C]
1'	1.25	1.12	6.72	15.4	2.59
2'	0.84	8.14	2.23	1.81	0.96
3'	1.02	1.11	7.32	17.2	3.33
4'	1.49	8.14	2.81	2.40	2.38
5'	1.53	1.14	8.40	16.2	3.32
6'	1.10	7.47	3.04	1.84	1.13
7'	1.63	1.21	9.25	18.1	3.49
8'	1.47	8.17	3.15	2.87	0.93

thus the acetate pathway is removed from consideration. On the other hand, three carbons (C–2", C–6" and C–8") of *m*-aminoacetophenone are labeled by [6-^{13}C]glucose. Labeling of C–8" by [6-^{13}C]glucose is, presumably, as with 6-methylsalicylate, *via* acetate (16). Labeling of C–2" and C–6" is, however, precisely the result expected if *m*-aminoacetophenone is formed from a "C_7N" unit (11), since those are the carbons of shikimate derived from C–6 of glucose (17).

TABLE 4. Enrichment from labeled precursors.

Carbon	L-Met	Sodium acetate		D-Glucose	
	[CH_3-^{13}C]	[1-^{13}C]	[2-^{13}C]	[6-^{13}C]	[1-^{13}C]
1"	1.44	1.88	1.88	1.34	1.28
2"	1.27	1.12	1.05	32.7	3.63
3"	1.17	1.02	1.26	0.90	1.00
4"	1.32	1.39	1.14	1.84	1.56
5"	1.52	1.34	1.12	1.02	1.87
6"	1.47	1.06	1.62	21.5	5.17
7"	1.41	1.86	1.92	1.40	1.42
8"	1.82	1.42	12.3	25.4	3.58

Finally, the highly substituted cyclopentane nucleus of pactamycin is derived from a variety of sources (table 5). Acetate labels mainly the urea carbonyl in this part of the molecule, but the two *N*-methyl carbons and three carbons of the substituents on the cyclopentane ring (those in the α-hydroxyethyl and methyl groups) are derived from methionine. The latter three carbons are also labeled by C–6 of glucose, which is presumably converted to methionine *via* glycerate, serine, and tetrahydrofolate (16, 18). It is particularly remarkable that two contiguous carbons are methionine-derived, as in the 24-ethyl sterols. In addition, [6-^{13}C]glucose labels C–9 (the branching hydroxymethyl carbon on the cyclopentane ring), while C–3 of the cyclopentane ring is labeled by C–1 of glucose. Overall, then, the biosynthetic labeling of pactamycin can be summarized as in figure 4.

Beyond the labeling patterns *per se*, a number of questions remain to be answered. The first concerns the intermediates en route to *m*-aminoacetophenone.

TABLE 5. Enrichment from labeled precursors

Carbon	L-Met	Sodium acetate		D-Glucose	
	[CH_3-^{13}C]	[1-^{13}C]	[2-^{13}C]	[6-^{13}C]	[1-^{13}C]
1	1.01	0.81	0.85	0.81	1.99
2	1.14	1.11	0.88	0.97	1.29
3	1.17	0.86	0.94	0.98	8.44
4	1.20	1.03	0.92	1.81	1.09
5	1.09	1.11	0.99	1.09	1.07
6	23.1	1.21	2.81	17.5	3.34
7	20.3	1.05	2.42	16.3	3.30
8	23.7	1.22	2.85	18.1	3.48
9	1.62	1.03	1.04	34.5	3.27
10	0.93	7.71	5.48	6.37	2.01

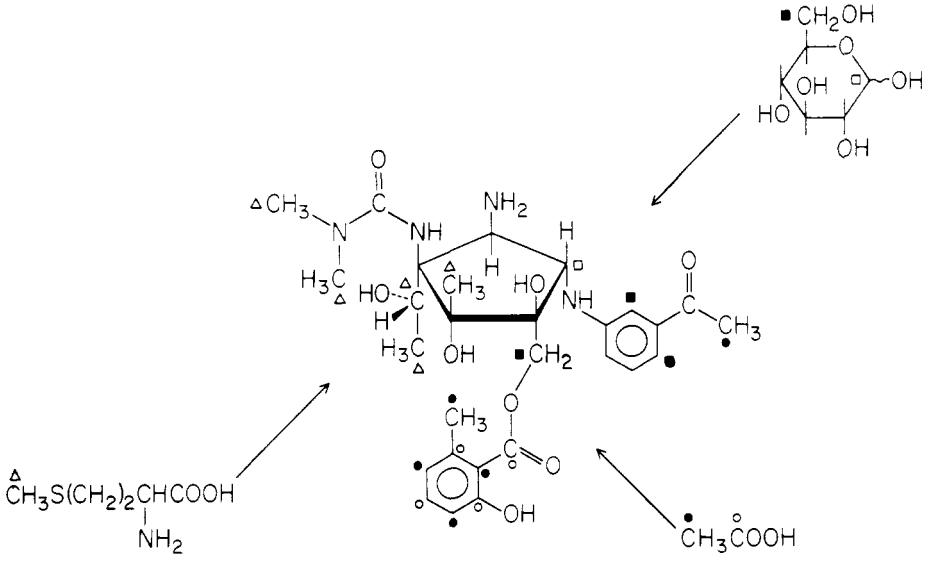


FIG. 4. Incorporation of methionine, glucose and acetate into pactamycin.

The origin of the "C_7N" unit seems, as noted above, to be related to that of the aromatic amino acids, in that C-6 (and, to a lesser extent, C-1) of glucose labels two carbons. Moreover, the labeling is unequal, with the C-2" and C-6" carbons being labeled to greater and lesser extents (C-2" > C-6" by [6-^{13}C]glucose), C-6" > C-2" by [1-^{13}C]glucose), in ratios similar to those observed in the biosynthesis of shikimic acid (17). This suggests that the amino group becomes attached to the carbon of shikimic acid which is converted to a carbonyl group in dehydroshikimate and dehydroquinate (fig. 5). Which, if either, of the latter two intermed-

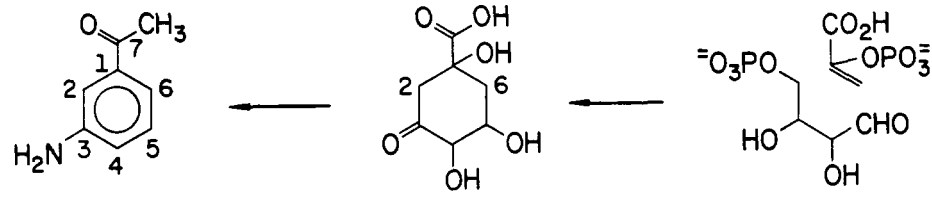


FIG. 5. Labeling of C-2 and C-6 of *m*-aminoacetophenone by C-6 of glucose via phosphoenolpyruvate and erythrose-4-phosphate.

iates is, in fact, converted to the "C_7N" unit is the subject of continuing investigations.[2]

The question of the route to the cyclopentane unit is also under continuing investigation. Two plausible alternatives exist (fig. 6). Glucose may be converted directly to a hydroxymethyl-substituted cyclopentane by cyclization of C-1 of glucose onto C-5 in a reaction analogous to an aldol cyclization, triggered

[2]Recent results on mitomycin biosynthesis (19) argue in a contrary direction—that the amino group is attached to a carbon on the opposite side of the "C_7N" unit and those authors suggested that the amino group is introduced prior to cyclization.

perhaps by a carbonyl group either at C–4 or at C–6. This reaction scheme would be like that involved in the conversion (20) to inositol or inosose, which are intermediates in the biosynthesis of streptidine (21). Alternatively, cyclization could proceed through an inosose itself, followed by a rearrangement in which the previous C–6 of glucose is expelled much in the same manner as C–3 of 6-deoxy-*D-xylo*-4-hexosulose in its rearrangement leading to L-dihydrostreptose (22).

Our studies on the biosynthesis of pactamycin have led us to reexamine the fermentation broth of *Streptomyces pactum* var. *pactum*, where a number of minor components related to pactamycin have been found to be co-produced. Structures of these minor components (**8–13**) are related to pactamycin either by hydroxylation or deoxygenation; they were assigned from the components' field desorption mass spectra and by comparison of their ^{13}C nmr spectra to those of pactamycin itself; the shift method was used to locate additional hydroxylation or deoxygenation. 8''-Hydroxypactamycin (**8**) can be presumed to be a metabolite of pactamycin arising from hydroxylation. 7-Deoxypactamycin (**9**), however, is presumably a precursor of pactamycin which becomes oxygenated at a later stage. This conclusion is supported by the presence of the epimer, 7-epipactamycin (**10**). Investigation of the relative bioactivities of these compounds indicates that the microbiological activity of the pactamycin-related compounds (**8–10**) is similar to that of pactamycin itself, but the pactamyçate-related compounds (**6**, **11–13**) are not active as antibacterial agents.

BERNINAMYCIN.—The second compound whose biosynthesis will be discussed belongs to a different class of antibiotics, each of which is characterized by the presence of one or more dehydro amino acids. A partial list indicating the dehydro amino acids present in such antibiotics and other compounds is found in table 6.

The origin of dehydro amino acids has been the subject of some speculation through the years (54). There are at least three different possibilities for the source of these compounds, as can be illustrated by the formation of dehydroalanine (fig. 7). All involve incorporation of an amino acid into the polypeptide, followed by conversion of the peptide-bound amino acid to the peptide-bound dehydro amino acid. One mechanism involves the incorporation of alanine into the polypeptide, followed by its dehydrogenation to the dehydro amino acid peptide. A second involves dehydration of peptide-bound serine, and a third possibility involves the loss of hydrogen sulfide from peptide-bound cysteine to give the dehydro amino acid.

Our own study of the formation of dehydro amino acids has centered on the antibiotic berninamycin, a potent inhibitor of gram-positive organisms (MIC 0.2 μg/ml vs. *B. subtilis*) (55), whose mode of action appears to be the inhibition of protein synthesis (56). The structure of berninamycin was assigned recently from our laboratory (43) and is shown below (**14**).

More recently still, questions have been raised concerning the nature of the most unusual portion of berninamycin, the polycyclic unit, which we have called berninamycinic acid. Hydrolysis of berninamycin in aqueous acid at 110° gives berninamycinic acid (**15**), whose structure was established by X-ray diffraction (57). The structural unit corresponding to **15** in **14** would be **a** (fig. 8). Berninamycinic acid was isolated later from another antibiotic, sulfomycin, by Abe, *et al.*

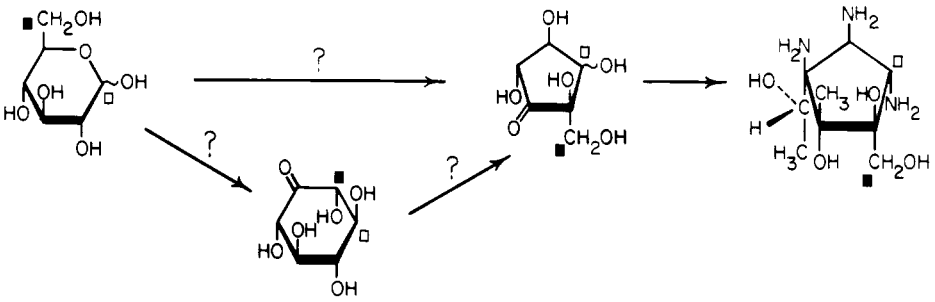
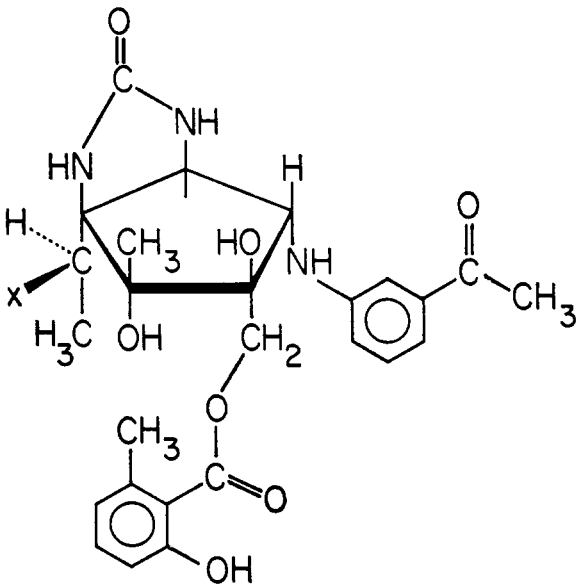


FIG. 6. Possible routes for bioconversion of glucose to the cyclopentane unit of pactamycin.



12 : x = H

13 : x = OH

TABLE 6. Dehydro amino acid containing compounds.

Antibiotics	Dehydro analog of	Reference (structure)
Mycelianamide	Tyr	23–25
Thiostrepton	But, Ala, Cys	26, 27
Virginiamycin M	Pro	28
Teleomycin and antibiotics LL-AO 341A, LL-AO 341B, A-128 OP, and A-128 P	Try	29–31
Stendomycin	But	32
Siomycins A, B, C	Ala	33
Nisins	Ala, But	34
Viomycin, capreomycins A and B, tuberactinomycins A, N, and O	Diaminopropionic acid	35–40
Subtilin	Ala, But	41
Nosiheptide	Ala	42
Berninamycin	Ala, But, Cys	43
Thiopeptin	Ala	44
Sulfomycin	Ala	45
Griseoviridin	Diaminopropionic acid (modified)	46
Cephalosporins	Val (modified)	47
Other compounds		
3,6-Dibenzylidene-2,5-dioxopiperazine and 3-benzyl-6-benzylidene-2,5-dioxopiperazine	Phe	48
Albonoursin	Phe, Leu	49
Austamide	Pro	50
Tentoxin	Phe	51
Tryptophan-dehydrobutyrine diketopiperazine	But	52
Alternariolide	Ala	53

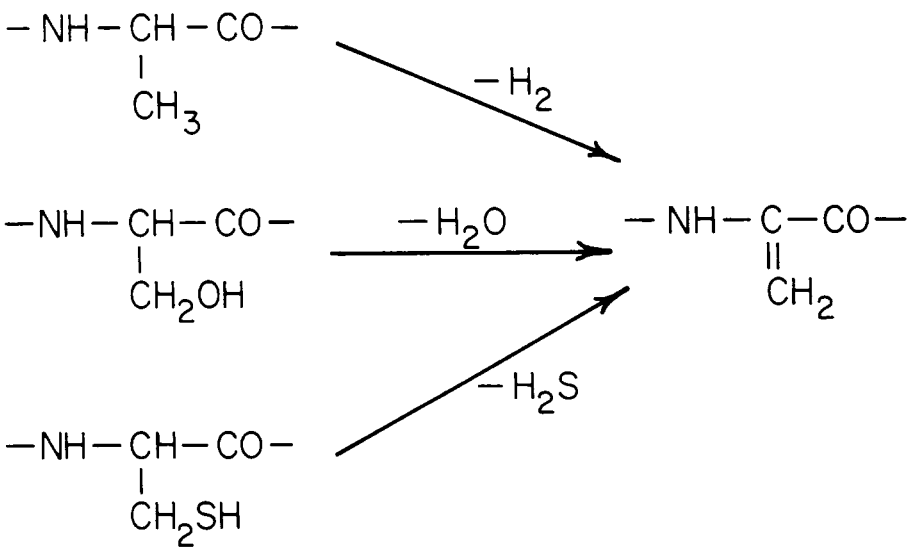
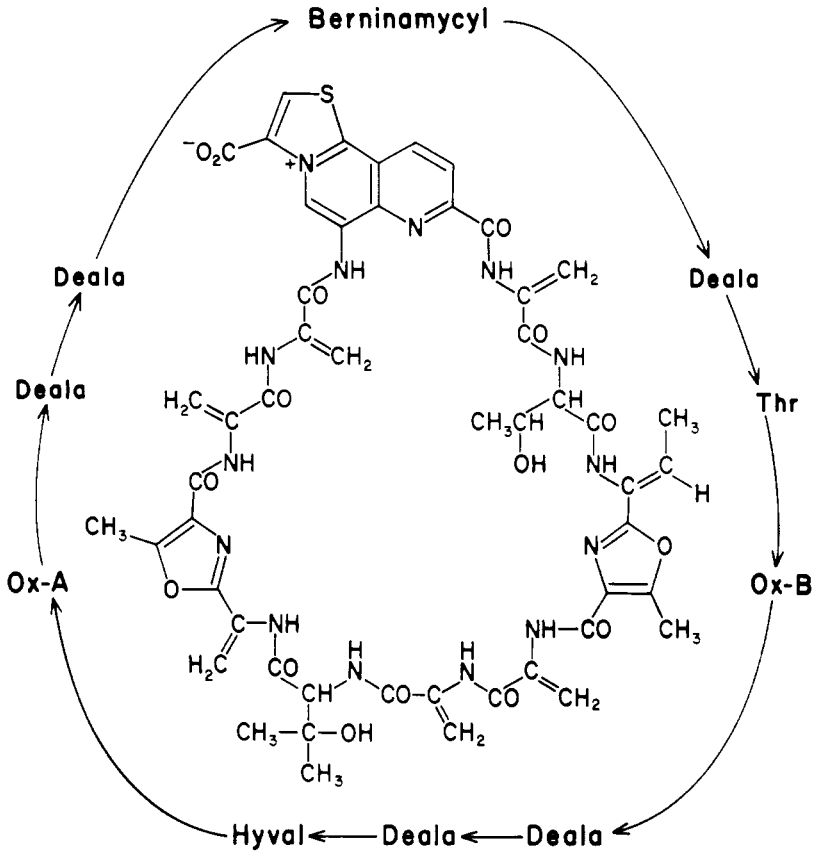


FIG. 7. Hypothetical routes from peptide-bound amino acids (Ala, Ser, Cys, top to bottom) to dehydro amino acids.



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(58). Later still, the same group reported that methanolysis of sulfomycin did not give berninamycinic acid but, rather, dimethyl sulfomycinamate (**16**) (45), which could be converted to **15** in aqueous acid. These workers suggested the presence of the sulfomycinamyl unit (**b**) rather than unit **a** in berninamycin and sulfomycin. This point has not yet been resolved, and a third alternative (**c**) is also possible.

The biosynthesis of the berninamycinic acid (or sulfomycinamic acid) unit in berninamycin and sulfomycin is of interest regardless of its structure. If we assume for the present that the structural unit involved is related to berninamycinic acid, two possibilities exist for its biosynthetic origins. The first possibility, as shown in fig. 9, involves the incorporation of one mole of cysteine (Cys), one mole of serine (Ser, or cysteine or alanine), and one mole of α-aminoadipic acid (AAA) into the three-ring unit; the second possibility (also shown in fig. 9) involves the incorporation of one mole of cysteine, one mole of aspartic acid (Asp), and one mole of glutamic acid (Glu).

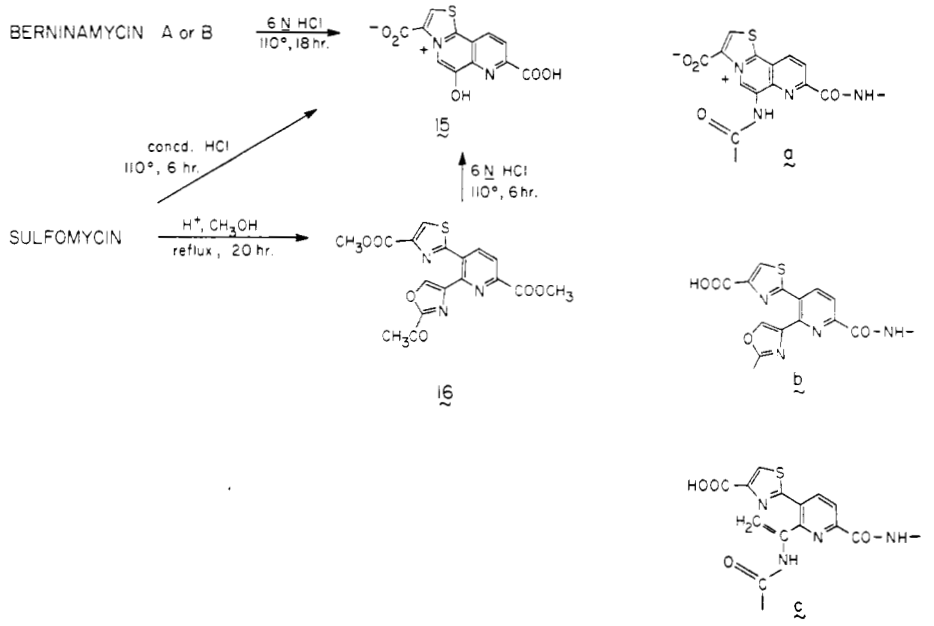


FIG. 8. Hydrolysis of berninamycin and sulfomycin to berninamycinic acid (**15**) and sulfomycinamic acid (**16**). Possible units of the antibiotics are shown as **a, b** and **c**.

An additional problem to be solved in berninamycin biosynthesis, in addition to those involving the origins of the dehydroalanine and berninamycinic acid units, concerns the origin of the oxazole units. A portion of oxazole A is presumed to be derived from the same source as the dehydroalanine units, with oxazole B and the remainder of oxazole A coming from threonine or butyrine.

As shown in table 7, a number of potential precursors for berninamycin have

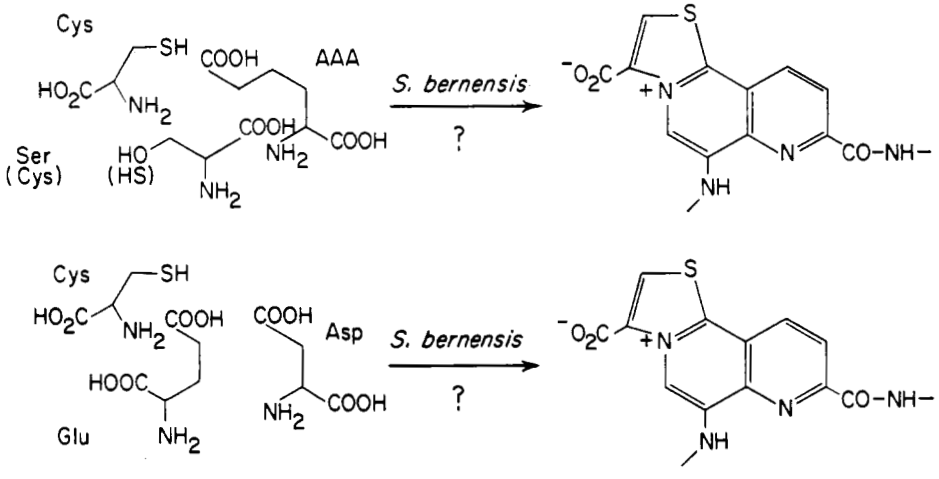


FIG. 9. Alternative sets of biosynthetic precursors of the berninamycinic acid unit of berninamycin.

TABLE 7. Incorporation of ^{14}C labeled precursors.

	% Incorporation, berninamycin	Specific activity, μCi/mmole		
		Berninamycin	Berninamycinic acid	Pyruvate DNP hydrazone
L-[U-^{14}C]Ser	2.6	0.369	0.110	0.061
D,L-[1-^{14}C]Ser	1.9	0.384	0.129	0.056
D,L-[1-^{14}C]Ala	0.018	ND[a]	ND	ND
L-[U-^{14}C]Cys	0.27	0.031	0.039	0.0013
D,L-[6-^{14}C]AAA	0.045	ND	ND	ND
D,L-[4-^{14}C]Asp	0.008	ND	ND	ND
D,L-[1-^{14}C]Glu	0.000	ND	ND	ND
L-[U-^{14}C]Lys	0.24	ND	ND	ND
L-[U-^{14}C]Thr	0.121	ND	ND	ND
D,L-[1-^{14}C]But	0.007	ND	ND	ND
D,L-[1-^{14}C]Val	0.20	ND	ND	ND
L-[*methyl*-^{14}C]Met	0.002	ND	ND	ND
D-[U-^{14}C]Glucose	0.065	ND	ND	ND

[a]Not determined.

been administered to *Streptomyces bernensis*, the organism producing the antibiotic. From the results in table 7, it is clear that the dehydrogenation pathway is not the pathway followed for the production of the dehydroalanine units, since alanine itself was not incorporated into berninamycin. On the other hand, the

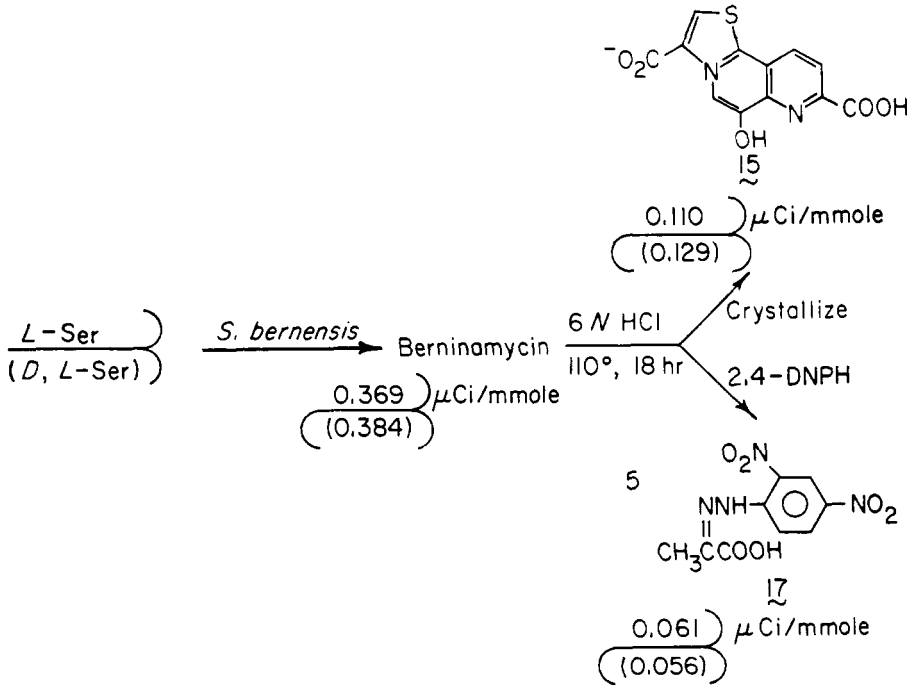


FIG. 10. Incorporation of serine into berninamycin and its berninamycinic acid (**15**) and dehydroalanine units. The latter is isolated as pyruvic acid dinitrophenylhydrazone (**17**).

two remaining potential precursors of the dehydroalanine unit, cysteine and serine, were both incorporated; thus it is necessary to distinguish between those two pathways. This was done by hydrolyzing berninamycin to berninamycinic acid and pyruvic acid and comparing the radioactivities of the degradation products with that of the intact antibiotic. The degradation scheme is shown in fig. 10.

Table 7 indicates that the specific activity of berninamycinic acid (**15**, 0.039 μCi/mmole) from labeled cysteine is the same as that of berninamycin (0.031 μCi/mmole); hence, cysteine cannot be the origin of the dehydroalanine units. On the other hand, the radioactivity of berninamycin derived from serine is found (table 7) in both berninamycinic acid and in the dinitrophenylhydrazone of pyruvic acid (**17**), the other principal degradation product from vigorous acidic hydrolysis. Since five moles of pyruvic acid are formed by hydrolysis of one mole of berninamycin (59) and only one mole of berninamycinic acid, the specific activity of berninamycin attributable to the two degradation products is five times the specific activity of pyruvic acid plus the specific activity of berninamycinic acid, a sum which is close to the specific activity of berninamycin itself. Thus, the origin of the dehydroalanine units in berninamycin seems clearly established as serine.

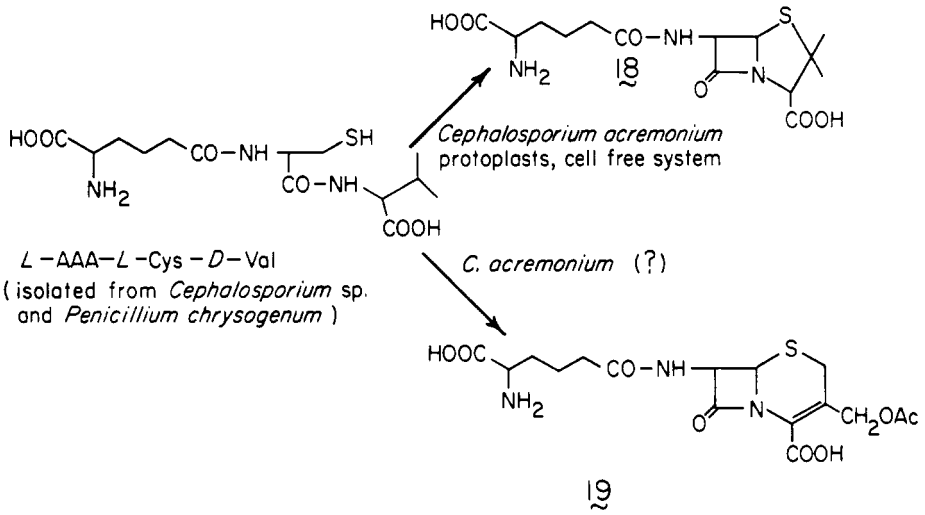


FIG. 11. Origin of penicillin N (**18**) and cephalosporin C (**19**) from a tripeptide containing aminoadipic acid and cysteine.

The activities in table 7 give some clue to the origin of other units in the molecule as well. Threonine can be seen to be well incorporated, and can be presumed to be the origin of the threonine and oxazole (Ox-A, Ox-B) units with the exception of that portion of oxazole B containing a hidden dehydroalanine unit. The incorporation of valine can be presumed to be into the β-hydroxyvaline (Hyval) unit of the antibiotic.

Finally, the incorporation of α-aminoadipic acid is considerably better than that of aspartic acid or glutamic acid; thus, α-aminoadipic acid is likely to be converted to the pyridine ring of berninamycinic acid. Better incorporated still is lysine, so that the more direct precursor may, in fact, be the ϵ-semialdehyde of α-aminoadipic acid, into which both lysine and α-aminoadipic acid can be converted (60).

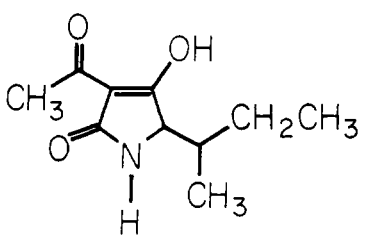
If α-aminoadipic acid is indeed a precursor of berninamycinic acid, this raises the interesting possibility of a relationship between berninamycin on the one hand and penicillin N (**18**) and cephalosporin C (**19**) on the other, since both **18** and **19** appear to be derived from a tripeptide in which an α-aminoadipyl-cysteinyl bond is present, as shown in fig. 11 (60a).

STREPTOLYDIGIN.—A final example of the biosynthesis of unusual antibiotics is found among the acyl tetramic acids. Although not as numerous as the dehydro amino acid-containing antibiotics, the acyl tetramic acids constitute a distinct and reasonably large group of antibiotics and related compounds (table 8). Bio-

TABLE 8. Acyl tetramic acid antibiotics (partial list).

Antibiotic	Reference (structure)
Tenuazonic Acid	61
Streptolydigin	62
Erythroskyrine	63
Cyclopiazonic Acid	64
Tirandamycin	65
α-Lipomycin	66
Ikarugamycin	67
K16	68
Oleficin	69
Magnesidin	70
Equisetin	71

syntheses of the three compounds shown below, all produced by microorganisms, have been investigated. Tenuazonic acid (**20**) is an antitumor agent, erythroskyrine (**21**) and cyclopiazonic acid (**22**) are mold toxins. Stickings established



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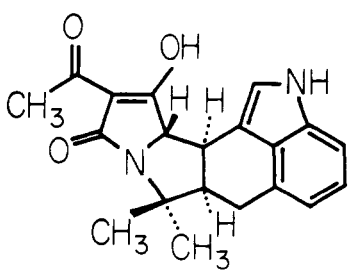

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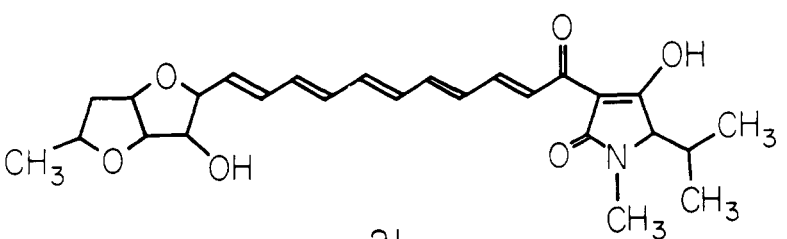

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that isoleucine is incorporated into tenuazonic acid, together with two moles of acetic acid (72). This general biosynthetic pathway (figure 12) involving an amino acid and several moles of acetate or malonate, sometimes plus other units, has been confirmed by Shibata for erythroskyrine (73) and by Holzapfel for cyclopiazonic acid (74).

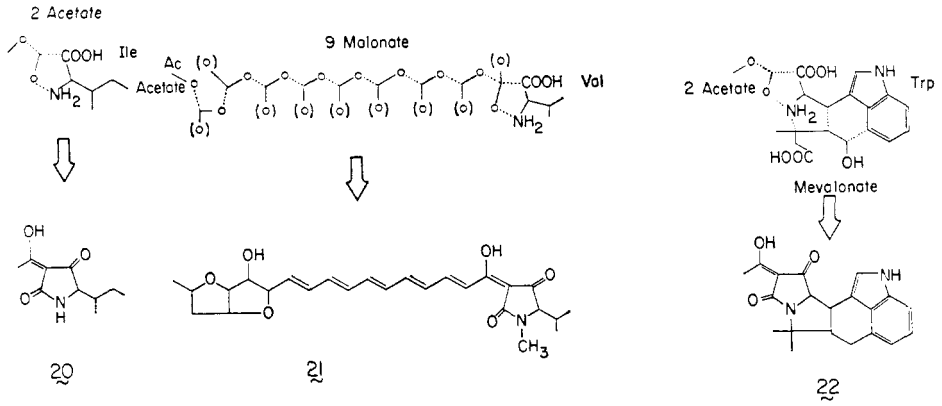
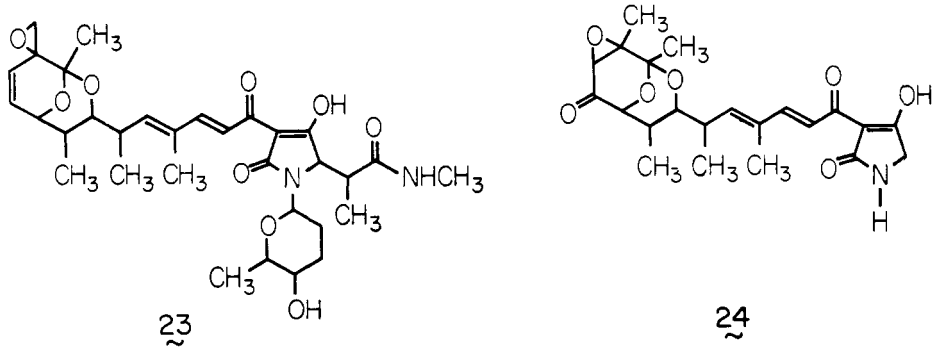


FIG. 12. Biosynthetic origins of tenuazonic acid (**20**), erythroskyrine (**21**) and cyclopiazonic acid (**22**).

Our own attention has been drawn for some time to two other acyl tetramic acids, streptolydigin (**23**) and tirandamycin (**24**), whose structures we assigned in 1963 (62) and 1971 (65). Streptolydigin is the more useful antibiotic, with potent activity against gram-positive organisms (75); both inhibit bacterial RNA polymerase by inhibiting chain elongation (76–80). Interest has been stimulated recently in these compounds by their inhibition of terminal deoxyribonucleotidyl transferase (81), an enzyme considerably more abundant in tumor cells than in other mammalian cells.



Each of the antibiotics can be converted by periodate oxidation to the corresponding carboxylic acid, streptolic acid (**25**) from streptolydigin and tirandamycic acid (**26**) from tirandamycin. Conversion of the two acids to a common degradation product (**27**), as shown in fig. 13, established their identical stereochemistry, which has been determined by an x-ray study of a tirandamycic acid derivative.

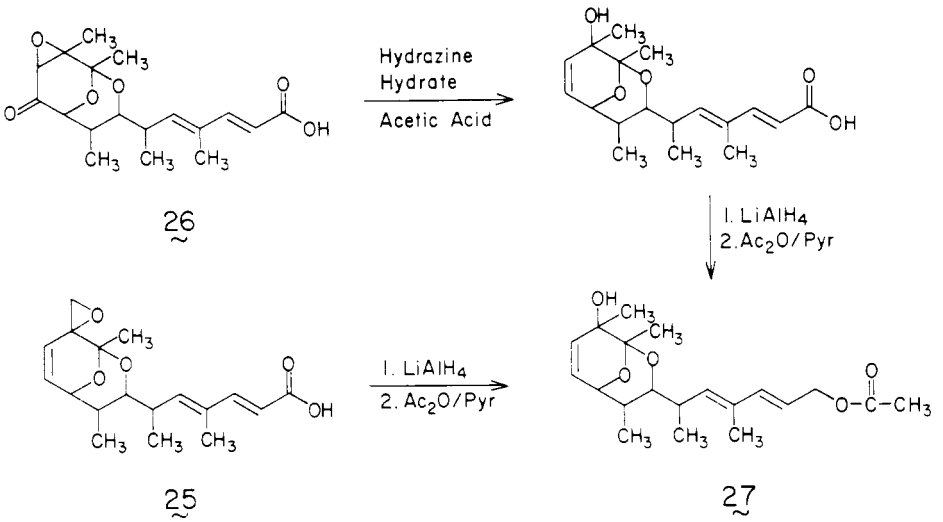


FIG. 13. Conversion of streptolic acid (**25**) and tirandamycic acid (**26**) to a common degradation product (**27**).

Biosyntheses of the two compounds can be predicted with a reasonable degree of confidence up to a point, in that glycine should provide a portion of the tetramic acid ring of tirandamycin (**24**) and β-methylaspartic acid a portion of the tetramic acid ring of streptolydigin (**23**). Like other deoxy sugars, the one in streptolydigin is presumably derived from a hexose, perhaps glucose.

Interest in the biosynthesis centers on the origin of the streptolic acid and tirandamycic acid portions of the molecules, which should be constructed from identical precursors. While it is clear that the side chain must stem from a polyketide, it is not clear from inspection whether the methyl groups originate from methionine units attached to a polyketide chain or from propionate units which form part of the polyketide. Precedence can be cited for both postulates. The origin of the methyl groups of most macrolide antibiotics is propionate (82), but no branched-chain acyl tetramic acid has been investigated previously. On the other hand, the biosynthetic work of Tamm, *et al.* on the cytochalasins (fig. 14) argues for methylation of a polyketide chain, since the methyl groups of cyto-

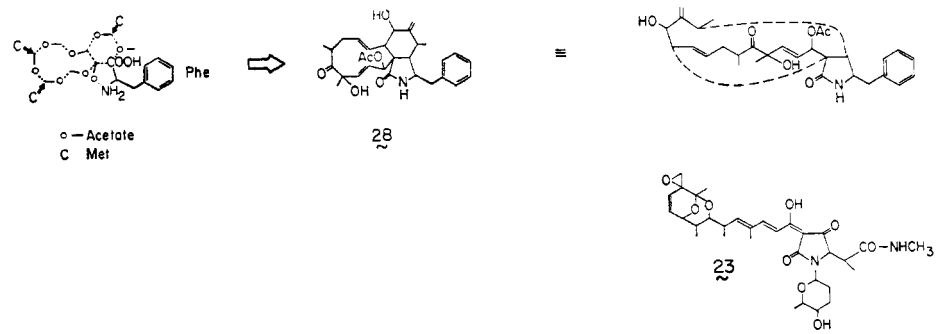


FIG. 14. Biosynthesis of cytochalasin D (**28**) and its hypothetic relationship to streptolydigin (**23**).

TABLE 9. Precursor incorporation into streptolydigin.

Precursor added	% Incorporation	% of Label in streptolic acid
[3-^{14}C]Propionate	0.72–1.6	100
[1-^{14}C]Acetate	0.024	ND[a]
L-[*methyl*-^{14}C]Methionine	0.24–0.48	1–3
D-[U-^{14}C]Glucose	0.29	ND
D,L-[1-^{14}C]Glutamate	0.01	ND
[1-^{14}C]Malonate	0.038	ND
D,L-[4-^{14}C]Aspartate	0.007	ND

[a]Not determined.

chalasin D (**28**), whose structure resembles that of a highly modified acyl tetramic acid derived from a polyketide of the same chain length as streptolydigin (**23**, fig. 14), are derived from methionine (83). The pyrrolidone ring in cytochalasin D is, in part, derived from phenylalanine, providing a parallel with tetramic acid rings, which are derived in part from amino acids, as noted above.

To settle this question, a variety of ^{14}C-labeled precursors have been administered to *Streptomyces lydicus*, the producer of streptolydigin, as summarized in table 9. From this table, it is clear that both propionate and methionine, as well as glucose, are incorporated well into streptolydigin. However, periodate oxidation gives streptolic acid in which essentially all of the label from propionate is found in streptolic acid but none of the label from methionine. Thus, propionate rather than methionine provides the methyl groups of streptolydigin; and

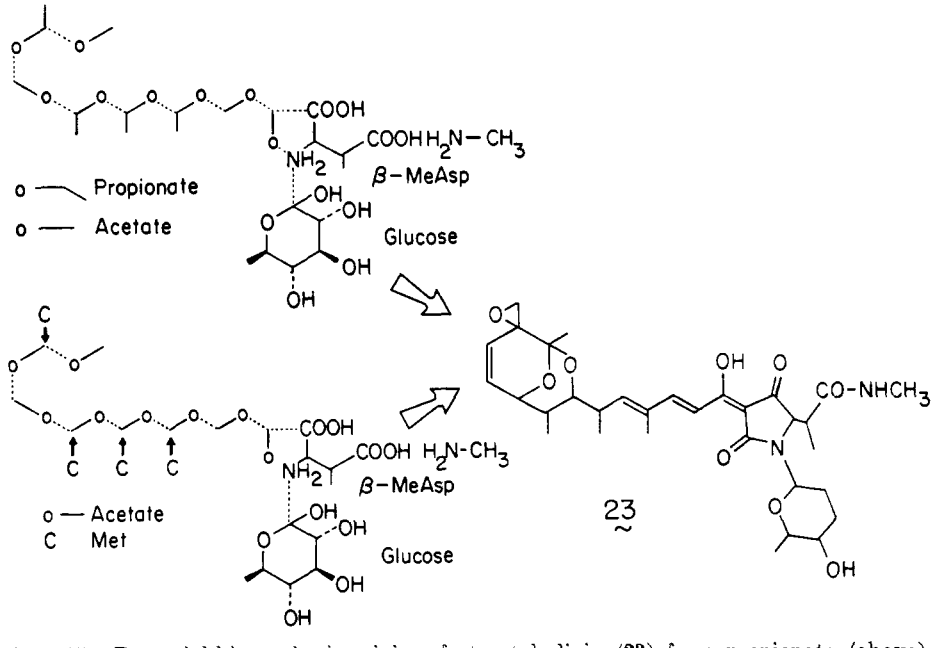


FIG. 15. Potential biosynthetic origins of streptolydigin (**23**) from propionate (above) and methionine (below).

methionine, presumably, labels the methylamino carbon.[3] Although our biosynthetic studies of streptolydigin are far from complete, it is already clear that, although streptolydigin and cytochalasin D are superficially related, their biosynthetic pathways differ at the most fundamental level, i.e., in the origin of the carbon skeleton. Our present view of the biosynthesis is shown in fig. 15.

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[3]Recent work in our laboratory employing [*methyl*-^{13}C]methionine and [*carboxy*-^{13}C]propionate substantiates the hypotheses based on ^{14}C data (84).

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