Tetrahedron. Vol 32, pp. 217 to 222 Pergamon Press 1976 Printed in Great Britain

SPECTINABILIN, A NEW NITRO-CONTAINING METABOLITE ISOLATED FROM *STREPTOMYCES SPECTABILIS*†

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(*Received in the USA 7 April* 1975; *Received in the UK for publication 18 August* 1975)

Abstract—A new nitro-containing metabolite, spectinabilin, was isolated from the crude streptovaricin complex produced by *Streptomyces spectabilis*. The structure was determined by degradation studies and spectral properties. A possible biosynthetic pathway of the metabolite is discussed in relation to the biosynthesis of the streptovaricins.

The streptovaricins produced by *Streptomyces spectabilis* constitute a group of the most important ansamycin antibiotics,[1] showing strong antibacterial activity, especially against Mycobacteria, as well as inhibition of bacterial DNA directed RNA polymerase and RNA dependent DNA polymerase (reverse transcriptase, RT) of tumor viruses. However, it has become apparent recently that crude streptovaricin complex contains other compounds of interest either for their biological activity or their structural or biosynthetic relationships to the streptovaricins. We report here on one of these novel compounds, spectinabilin, to which we assign the structure **1**.‡

Structural assignment. Spectinabilin was isolated from streptovaricin line product by silica gel column chromatography as a non-polar component whose chromatographic properties resemble most closely those of streptovaricin E,[2] the least polar streptovaricin. However, its pale yellow color is slightly different from the orange-yellow color of the streptovaricins. Spectinabilin is a weak inhibitor of RT, giving 52% inhibition of RLV§ reverse transcriptase at 200 μg/ml. The compound showed no anti-microbial activity. Spectinabilin is not very stable, about 50% being converted to other substances during 1 month at room temperature.

Yellow crystalline spectinabilin has the molecular formula $C_{28}H_{31}NO_6$, established by elemental analysis and high resolution mass spectrometry. Its electronic spectrum [λ_{max}^{EtOH} 218 nm (ϵ 19,100), 252 nm (ϵ 17,600), 268 nm (ϵ 18,200), 367 nm (ϵ 15,500)] is not altered in alkaline solution and is quite different from that of the streptovaricins. Lack of hydroxyl and amine protons is indicated by the PMR spectrum (Table 1), since deuterium oxide addition does not affect any signal even in the presence of trifluoroacetic acid.

†Presented in part at the 13th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., 19–21 Sept. 1973, Abstract No. 86.

‡In the structures assigned to **1** and **4** the all-*trans* stereochemistry shown for the tetraene system is speculative.

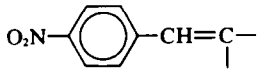
§Rauscher leukemia virus.

Table 1. PMR assignments $\delta^{b,c}$, m[d] (J, Hz) of **1**, **2**, **3** and **4**

Protons Location,[a] type	1[e]	2[e,f]	3[e,f]	4[e]
1a, $-OCH_3$	3·99,s	3·98,s	3·93,s	
2a, $-CH_3$	1·86,s	1·74,s	1·77,s	
4a, $-CH_3$	2·04,*s	1·90,s	1·91,s	
6, $-\overset{\vert}{C}H-O-$	5·20,t (7)	4·84,m	5·00,dd (7·9)	4·55,t (7)
7, $-CH_2-$	3·04,qd (7·15)	---[g]	---[g]	2·90,m
8,		---[g]	---[g]	
8a, $-CH_2-O-$	4·86,qb (13)	3·34,m 3·84,m	3·50,m 4·04,m	4·75,m (14)
9, =CH–	6·54,†s	---[g]	---[g]	6·45,*s
10,		---[g]	---[g]	
10a, $-CH_3$	2·06,*s	0·86,*d (7)	0·84,*d (7)	1·96,†s
11, =CH–	6·13,‡s	---[g]	---[g]	6·00,*s
12		---[g]	---[g]	
12a, $-CH_3$	2·09,*s	0·89,*d (7)	0·85,*d (7)	2·02,†s
13, =CH–	6·08,‡s	---[g]	---[g]	6·00,‡s
14		---[g]	---[g]	
14a, $-CH_3$	2·13,*s	0·95,*d (7)	0·91,*d (7)	2·08,†s
15, =CH–	5·92,†s	---[g]	---[g]	5·83,‡s
17, =CH–	7·51,d (9)	7·30,d (9)	6·75, d (8·5)	7·40,d (9)
18, =CH–	8·02,d (9)	8·07,d (9)	6·45,d (8·5)	8·17,d (9)

[a] See Fig. 1 for numbering. [b] ppm from TMS. [c] $CDCl_3$ solution. [d] Multiplicity: s, singlet; d, doublet; t, triplet, q, quartet; b, broad; m, multiplet. [e] Assignments marked *, †, and ‡ in any column are interchangeable. [f] Mixture of stereoisomers. [g] Could not be assigned because of complex signals.

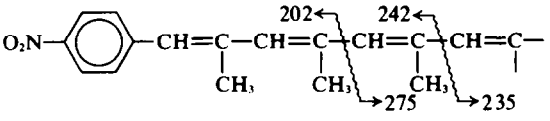
A *para*-disubstituted benzene was first suggested by an AB pair of doublets (J = 9 Hz) at 7·51 ppm (2H) and 8·02 ppm (2H) in the PMR spectrum of **1** (Table 1). One of the two *para* substituents was identified as a nitro group by IR absorption at 1520 and 1340 cm^{-1}, and ozonolysis of spectinabilin yielded *p*-nitrobenzaldehyde; thus, partial structure **a** was established.



a

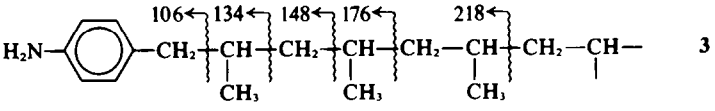
The UV maximum of spectinabilin at 367 nm requires that the compound be at least a *p*-nitrophenyltriene (since *p*-nitrophenylstyrene has λ_{max} 309 nm)[3] and would also agree with its formulation as a 1 - (*p* - nitrophenyl) - tetraene if the tetraene system were not completely coplanar. In agreement with the tetraene hypothesis, spectinabilin was converted on hydrogenation over Pd-C (Fig. 1) to a mixture of octahydrospectinabilin (**2**, $C_{28}H_{39}NO_6$) and octahydrospectinabilamine (**3**, $C_{28}H_{41}NO_4$), whose molecular formulas were assigned by mass spectrometry. These two products differ in that **3** is the *p*-aminophenyl analog of the *p* - nitrophenyl - containing **2**. Thus, **1** and **2** are negative to Dragendorff's reagent, **3** is positive; the nitro group absorptions remained for **2**, at 1530 and 1360 cm^{-1}, but were replaced by a characteristic amino group absorption at 3400 cm^{-1} in the IR spectrum of **3**. The AB signals appeared at 7·30 and 8·07 ppm (J = 9 Hz) in the PMR spectrum of **2** but were shifted to 6·45 and 6·75 ppm (J = 8·5 Hz) in the spectrum of **3**.

More importantly, the UV spectrum of **2** [λ_{max}^{EtOH} 247 nm (sh, ϵ 24,300), 268 nm (ϵ 31,400)] no longer contained the max at 367 nm and had lost four olefinic proton singlets (5·92, 6·08, 6·13, 6·54 ppm) and three olefinic methyl singlets (of the five at 1·86, 2·04, 2·06, 2·09 and 2·13 ppm) found in the PMR spectrum of **1**. In view of the singlet character of the olefinic proton signals, only two patterns are allowed, **b** and **b'**, viz. **b**: Ar–CH=C(CH=C)–CH=C(CH=C)–CH=C(CH=C)– or **b'**: Ar–CH=C(CH=C)–C(=CH–)–CH=C(CH=C)–C(=CH–). A decision between these options is reached in favor of **b** and the three olefinic Me groups are placed by the mass spectrum of **1**, which contains peaks at *m/e* 202, 235, 242 and 275, corresponding to the fragmentations shown for **c**:



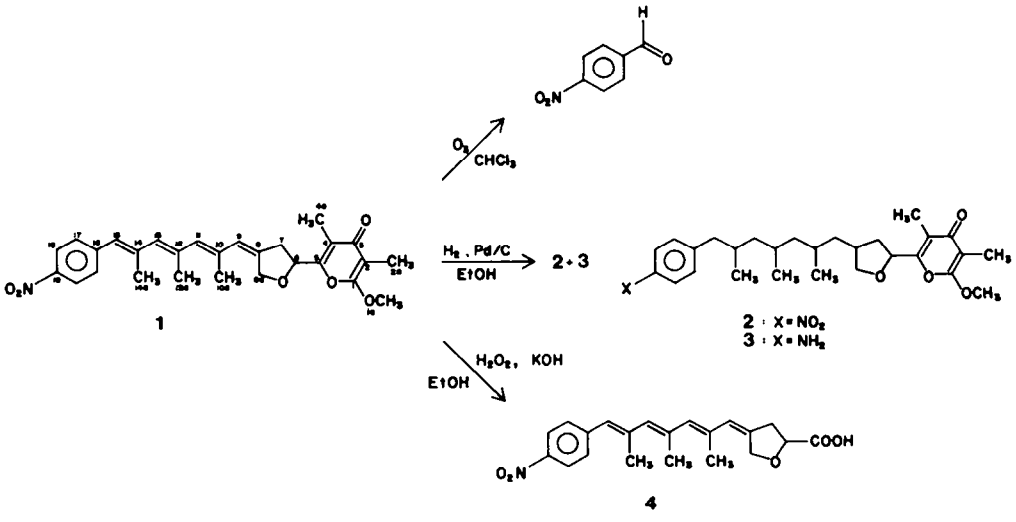
c

These cleavages are corroborated by ions in the mass spectrum of **3** at *m/e* 106, 134, 148, 176 and 218, as shown:



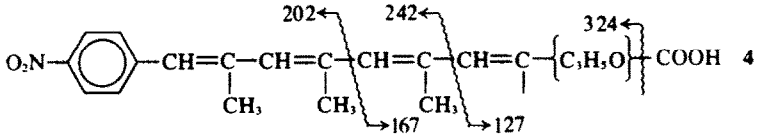
3

Oxidation of spectinabilin with hydrogen peroxide in alkaline solution (Fig. 1) afforded the yellow spectinabilic acid (**4**, $C_{21}H_{23}NO_5$) whose IR spectrum contained carboxyl absorption at 1725 cm^{-1} and nitro absorption at 1540 and 1340 cm^{-1}. The UV spectrum of **4** [λ_{max}^{EtOH} 248 nm (ϵ 14,000), 283 nm (ϵ 15,100), 358 nm (ϵ 12,100)] resembled that of **1** but lacked the maximum at 218 nm. The *p*-nitrophenyl group in **4** appeared in its PMR spectrum as a pair of doublets at 7·40 and 8·17 ppm; the four olefinic protons were observed as broad singlets at 5·83, 6·00 (2H) and 6·45 ppm. The mass spectrum of **4** contained a molecular ion at *m/e* 369 and fragment ions at *m/e* 324 (M − COOH) and *m/e* 242, 202, 167 and 127, as shown.



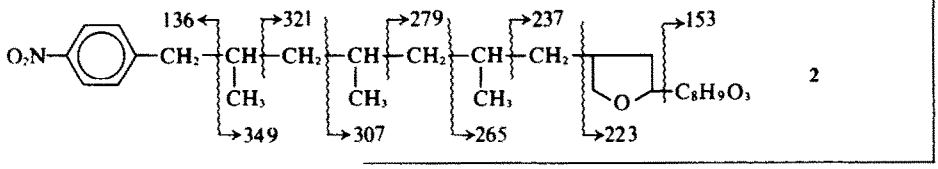
1 **4**

Fig. 1. Spectinabilin (**1**), its numbering scheme, reactions, and degradation products: octahydrospectinabilin (**2**); octahydrospectinabilamine (**3**); spectinabilic acid (**4**).



4

The remaining five protons of **4** were observed as an AB quartet (2 H, J = 14 Hz) centered at 4·75 ppm (=C–CH_2–O–), a multiplet (2 H) centered at 2·90 ppm and a methine triplet (J = 7 Hz) at 4·55 ppm (=C–CH_2–CH–C=). The multiplicity of the methylene protons indicates their presence in a ring, =C—CH_2–CH–; thus, the structure of **4** is as shown in Fig. 1. Oxidation of **1** to **4** converted a $C_8H_9O_3$ group to a carboxyl group. Additional confirmation of the $C_8H_9O_3$ group (153 amu) as well as of the positions of the Me groups is provided in the mass spectrum of **2**, which shows the ions indicated.



2

The $C_8H_9O_3$ group contains the remaining two olefinic Me groups (*vide supra*), a CO group (IR, 1670 cm^{-1}) and a OMe group which appears in the PMR spectrum of **1** at 3·99 ppm. Lack of ester CO absorption indicated the latter to be an enolic OMe. The third oxygen must be present in an ether linkage, since no OH groups are found in spectinabilin (*vide supra*). The formula of the $C_8H_9O_3$ group indicates four unsaturation elements; thus, in addition to the CO it must contain (a) a ring plus CH_3–C=C–CH_3 and CH_3O–C=C–; (b) a ring plus CH_3–C=C–OCH_3 and CH_3–C=C–; (c) the groups $(CH_3)_2$–C=C–OCH_3 plus –C≡C–; or (d) the groups CH_3O–C=C–CH_3 plus –C≡C–. An acetylene can be eliminated by the carbon magnetic resonance spectrum (*vide infra*). Incorporating the groups from possibilities (a) or (b) into a ring with an ether oxygen and a CO group requires either a pyrone or an acyl furan. The CO absorption at 1670 cm^{-1} and the UV absorption near 250 nm are inappropriate for an acyl furan. However, the UV spectra of **1**, **2** and **3** contain absorption appropriate for a γ-pyrone,[4] at 240–260 nm, as shown in Table 2. Moreover, the IR absorption of **1** in the CO region, at 1670 cm^{-1}, suggests a γ-pyrone system.[4] Neither the UV nor the IR data agree well with an α-pyrone structure.[5] The mass spectrum of spectinabilin also is in agreement with that expected for a γ-pyrone, with loss of two moles of carbon monoxide.[6] The γ-pyrone of **1**–**3** has two Me and one OMe substituents. Location of the OMe adjacent to the ether linkage, the usual position in γ-pyrones, is required by the upfield position of the pyrone C-2 carbon (99·1 ppm, *vide infra*) and is in agreement with the IR data of Table 2. The two Me groups are most appropriately placed at C-3 and C-5 of the pyrone and the alkyl chain at C-6, the only arrangement appropriate for the obvious propionate-acetate biosynthetic origin of spectinabilin. The complete structure of spectinabilin is then assigned as **1**.

Carbon magnetic resonance assignments. The structure of spectinabilin (**1**) assigned above was supported by its carbon magnetic resonance spectrum (Table 3). Signals were first grouped according to the multiplicities in the off-resonance proton-decoupled spectrum (i.e. assigned as methyl, methylene, methine or quaternary carbons) then further arranged according to standard chemical shift data.[7,8] Individual C atoms were assigned by comparison of the chemical shifts in **1** with absorptions of individual C atoms in the streptovaricins.[9]

Table 2. IR and UV absorptions of pyrones

Compound	IR (cm^{-1})	UV (nm)	Ref.
1	1670	252, 268	a
2	1675	247(sh), 268	a
3	1675	248	a
2-methoxy-3,6-dimethyl-4-pyrone	1670	260	4
2-methoxy-6-methyl-4-pyrone	1672	240	4
4-methoxy-3,6-dimethyl-2-pyrone	1690	300	5
4-methoxy-6-methyl-2-pyrone	1720	280	5

[a]Present data.

A characteristic CO signal at 180·6 ppm can be easily assigned to C-3 in the γ-pyrone.[10] α-Pyrone CO carbons are usually observed at 159–162 ppm.[11] This observation, therefore, further supports a γ-pyrone structure for **1**. C-1a, C-7, C-8a and C-6 are also determined clearly from

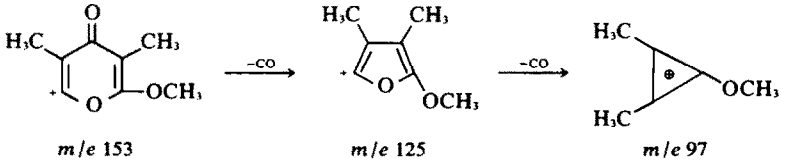


Table 3. Carbon magnetic resonance absorptions of spectinabilin

Group type	Chemical shift[a]	Assignment[b,c]
CH_3-	6·9	2a
	9·4	4a
	17·9	14a
	19·5	10a*
	19·6	12a*
-CH_2-	38·3	7
CH_3-O-	55·3	1a
-CH_2-O-	70·1	8a
-CH-O-	73·3	6
-CH=	126·8	15†
	128·1	9†
	123·5	18
	129·5	17
	134·4	13‡
	135·3	11‡
-C=	99·1	2
	119·9	4
	134·0	10**
	135·6	12**
	137·8	14††
	139·4	8††
	144·7	16
	145·9	19
-C=	155·2	5
O-	162·1	1
-C=O	180·6	3

[a]Ppm downfield from internal TMS. [b]The numbering system is shown in Fig. 1. [c]Assignments indicated *, **, †, ‡, and †† are interchangeable.

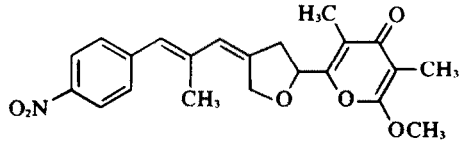
their unique combinations of off-resonance multiplicities and chemical shifts as the signals at 55·3, 38·3, 70·1 and 73·3 ppm, respectively.

The two Me carbons on the γ-pyrone are in a different region from the other Me carbons, shifted upfield by the ether oxygen of the ring to a location like that of toluquinone.[12] They are differentiated from one another by the effect of the second (OMe) oxygen. The signal at 6·9 ppm is thus assigned to C-2a and that at 9·4 ppm to C-4a. Assignments of the remaining three olefinic Me groups are tentative and interchangeable. However, the more separated Me carbon, at 17·9 ppm, is assigned to C-14a since C-10a and C-12a are in nearly identical environments.

The carbons of the *p*-nitrophenyl moiety are assigned by peak intensities and by calculations employing the known effects of aromatic substituents. Thus, C-17 and C-18 are assigned to 129·5 and 123·5 ppm since those peaks are of double intensity. They and the remaining benzene carbons (C-16 and C-19) are then assigned by comparison of the chemical shifts calculated for *p*-nitrostyrene from standard chemical shift data.[7] Thus, C-16 is found at 144·7 (calc. for C-1 of *p*-nitrostyrene, 144·2 ppm), C-19 at 145·9 (calc. 147·8 ppm), C-17 at 129·5 (calc. 127·5 ppm), and C-18 at 123·5 ppm (calc. 123·6 ppm). The olefinic quaternary carbons of the γ-pyrone ring are easily differentiated from other quaternary sp^2 carbons since they appear at both higher (C-2 and C-4) and lower (C-1 and C-5) fields than the others, due to the effects of the ring oxygen substituent. The extra oxygen substituent (OMe) shifts C-2 to higher field than C-4 and C-1 to lower field than C-5.

The remaining olefinic carbons are rather hard to assign. Among the hydrogen-bearing carbons, C-9 and C-15 should appear at similar chemical shifts as should C-11 and C-13, but the members of the pairs cannot be distinguished. Similarly, one cannot distinguish between the pairs of quaternary carbons (C-8 and C-14, C-10 and C-12). In general, the CMR signals of **1** are quite consistent with the structure assigned and further confirm it.

Related compounds and biosynthetic considerations. Compounds containing nitro groups are rather rare among natural products,[13] with only a few compounds having been reported, such as chloramphenicol,[14] aristolochic acid,[15] miserotoxin,[16] nitraminoacetic acid,[17] pyrrolnitrin,[18] azomycin[19] and the ilamycins.[20] Spectinabilin is structurally related to aureothin (**5**),[21] which is a toxic metabolite of *Streptomyces thioluteus*[22] and *S. luteoreticuli Arai*,[23] and to luteoreticulin (**6**), isolated from *S. luteoreticuli Arai*.[5,24]



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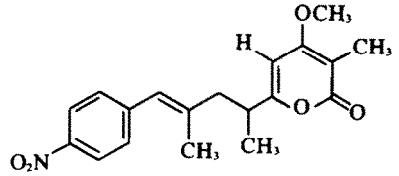
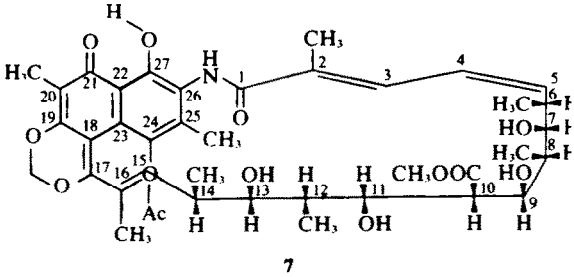


6

Spectinabilin contains two more propionate units than aureothin (**5**) and is presumably biosynthesized in the same manner as aureothin.[23,25]† The *p*-nitrobenzal group in spectinabilin should arise from *p*-aminobenzoate as an intermediate and, ultimately, from glucose through the shikimate pathway, like chloramphenicol.[26]

The structure of spectinabilin is also of considerable interest from the standpoint of streptovaricin biosynthesis.[27,28] Production of spectinabilin and streptovaricin by the same organism suggests possibly similar pathways for the two compounds. The streptovaricins, e.g. streptovaricin D (**7**), are biosynthesized by a propionate-



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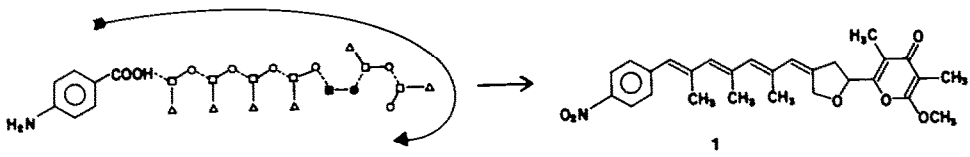
†Yamazaki, *et al.* studied the biosynthesis of aureothin (**5**), employing ^{13}C NMR.[23] However, they did not report the chemical shifts of all carbons, so the present assignments cannot be compared with those for aureothin in spite of the two compounds' similarity.



1

Fig. 2. Proposed biosynthetic origin of the carbon skeleton of spectinabilin (1).

acetate sequence in the same manner as aureothin (and presumably spectinabilin), with the synthesis proceeding away from the aromatic nucleus (amide-head direction) for streptovaricin,[27] as shown in Fig. 2. The remaining unit in the streptovaricins is a *m*-aminobenzoyl unit (with the aromatic Me group derived from methionine)[28b] and its origin appears to be glucose,[28a] perhaps through shikimate. *m*-Aminobenzoate is then a likely candidate starter for chain elongation in streptovaricin,[27] geldanamycin,[29] and rifamycin[30] biosynthesis. Recent work has shown that the C_7N (*m*-aminobenzoyl) unit of rifamycin B is derived from glucose.[31]

EXPERIMENTAL†

Isolation of spectinabilin (1). Streptovaricin complex (50 g, Upjohn, Lot 11560-4) was chromatographed on 1·1 kg of silica gel (Brinkmann) in a column 60 × 700 mm employing ethyl acetate–benzene (1:3) as eluant to give 3·7 g of a purple pigment followed by 4·4 g of crude spectinabilin. The column was further eluted with benzene–acetone solns to give mixtures of streptovaricins. The crude spectinabilin (2·8 g) fraction was purified on 300 g of silica gel (Brinkmann) in a column 45 × 600 mm employing $CHCl_3$–MeOH (99:1) as eluant. All of the eluted fractions were examined by TLC on silica gel GF-254 (0·25 mm thickness) employing $CHCl_3$–MeOH (98:2) as developing solvent. Appropriate fractions were combined and evaporated under reduced pressure to yield 2·3 g of spectinabilin, which was crystallized from acetone–hexane. The yellow crystals (840 mg) were soluble in acetone, chloroform, ether and methanol, insoluble in hexane, petroleum ether and water. Additional purification over a $\frac{3}{8}$" × 2' 100 Å μ Styragel high pressure liquid chromatography column gave the analytical sample, m.p. 91–92°; $[\alpha]_D^{26}$ +60·0° (*c* 5·0, $CHCl_3$), which showed a broad IR band (water of hydration) at 3410 cm^{-1}. Attempts to remove the water of hydration resulted in decomposition. (Found: C, 69·04; H, 6·74; N, 2·76; mol wt, 477·2115 (HRMS). Calcd for $C_{28}H_{31}NO_6$: C, 70·42; H, 6·54; N, 2·93; mol. wt., 477·2151. Calcd for $C_{28}H_{31}NO_6 \cdot 1/2H_2O$: C, 69·11; H, 6·62; N, 2·87%).

Ozonolysis of spectinabilin (1). Ozone was introduced into a soln of 1 (1·03 g) in 50 ml $CHCl_3$ at 0° until the yellow color disappeared; then the mixture was evaporated to dryness at room temp. under reduced pressure. The syrupy ozonide was decomposed by heating at 75° for 1 hr with 15 ml water. The water-insoluble material was collected by filtration and dissolved in a small amount of benzene. Solvent was evaporated to dryness *in vacuo* to give 310 mg brown solid, which was then sublimed at 75° (1·0 torr) to give 89 mg *p*-nitrobenzaldehyde, m.p. 105–107° (lit.[32] 106–107°), whose IR[33] and NMR[34] spectra were identical with those of an authentic specimen, obtained from Eastman Organic Chemicals, Rochester, New York.

Hydrogenation of spectinabilin (1). A mixture of 1·71 g of 1, 510 mg of 5% Pd–C, 30 ml EtOAc and 200 ml EtOH was stirred under H_2 at atmospheric pressure and room temp until H_2 absorption ceased. The mixture was filtered through celite on a glass funnel and reaction vessel, funnel and catalyst were washed thoroughly with EtOH. The filtrate was evaporated *in vacuo* to give an oily product, which was dried in a desiccator under vacuum to provide *ca.* 1·4 g of hydrogenated material containing two components (TLC), one of which gave a positive Dragendorff test and the other a negative. The product was chromatographed on 30 g of silica gel (Brinkmann) in a column 25 × 300 mm employing chloroform as solvent to give 283 mg of colorless oily *octahydrospectinabilin* (2), δ_{max}^{neat} 1675, 1625, 1600, 1530, 1470, 1430, 1380 cm^{-1}. (Found: C, 68·89; H, 7·82; N, 3·04; mol. wt. 485 (mass spec). Calcd. for $C_{28}H_{39}NO_6$: C, 69·25; H, 8·10; N, 2·88%; mol wt 485).

Continued elution with chloroform gave 274 mg of slightly brownish *octahydrospectinabilamine* (3) after 460 mg of a mixture of 2 and 3. The sample of 3 was further purified on 20 g of alumina (Merck) in a column 20 × 400 mm using chloroform as eluant to give 224 mg of chromatographically pure oily 3, δ_{max}^{neat} 3500, 3400, 1675, 1620, 1600, 1525, 1470, 1420, 1380, 1225 cm^{-1}. (Found: C, 73·13; H, 8·84; N, 2·86; mol. wt 455 (mass spec). Calcd. for $C_{28}H_{41}NO_4$: C, 73·81; H, 9·07; N, 3·07%; mol wt 455).

Spectinabilic acid (4). During 1·5 hr, 1 ml of 20% KOH in EtOH and 2·5 ml of 30% H_2O_2 soln were added simultaneously, dropwise with stirring at room temp. to 570 mg spectinabilin in 30 ml EtOH. The reaction was quenched by addition of 100 ml water and the aqueous soln was acidified to pH 3·0 with conc. HCl, then extracted twice with 100-ml portions ether. The combined ether extract was reextracted 3 times with sat $NaHCO_3$ aq. The resulting aqueous extract was again acidified with conc HCl, then extracted twice with 200-ml portions ether. The combined ethereal extract was washed with NaCl-saturated water and dried over Na_2SO_4. After filtration the ethereal soln was evaporated to dryness under reduced pressure to give 268 mg of yellow spectinabilic acid (4), m.p. 97–100°. (Found: mol. wt 369·1571 (HRMS). Calcd. for $C_{21}H_{23}NO_5$: Mol. wt 369·1576).

Acknowledgements—This work was supported by Contract NIH-NCI-C-72-3208 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. The high resolution mass spectrometer was purchased under grants from the National Cancer Institute (CA 11,388) and the National Institute of General Medical Sciences (GM 16864). The ^{13}C NMR spectrum was obtained on a spectrometer purchased with an instrumentation grant from the National Science Foundation. We thank the Upjohn Company for the sample of streptovaricin complex.

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