Biosynthesis of the 3,4-Dihydroxy-2,2-dimethyl-5-phenylvaleric Acid Residue of Neoantimycin

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The significance of the incorporation of [2-^{14}C]phenylalanine, [1-^{14}C]phenylalanine, [*Me*-^{14}C]methionine, and [2,3-^{3}H]propionate into the antibiotic neoantimycin is discussed in relation to the biosynthesis of the 3,4-dihydroxy-2,2-dimethyl-5-phenylvaleric acid residue.

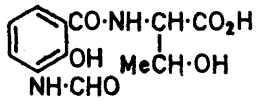
NEOANTIMYCIN, an antibiotic isolated from *Streptoverticillium orinoci*,[1] has been shown [2] to be a cyclic polyester resulting from condensation of the hydroxy-acids (I)—(IV). The present paper deals with the biosynthesis of fragment (IV), which has not previously been found in natural products.

The presence of the benzylic system prompted us to investigate the possibility of incorporation of labelled phenylalanine into neoantimycin. [2-^{14}C]Phenylalanine was added to a culture of *S. orinoci*. The resulting active neoantimycin, purified as the methyl ether, was hydrolysed under mild alkaline conditions, and the

[1] C. Cassinelli, A. Grein, P. Orezzi, P. Pennella, and A. Sanfilippo, *Arch. Mikrobiol.*, 1967, **55**, 358.

[2] L. Caglioti, D. Misiti, R. Mondelli, A. Selva, F. Arcamone, and G. Cassinelli, *Tetrahedron*, 1969, **25**, 2193.

fragment (IV) was isolated as the lactone (IVa), which contained the total radioactivity of the antibiotic. Reduction of the lactone (IVa) with lithium aluminium



(I)

$Pr^{i}CH(OH)\cdot CO_2H$

(II)

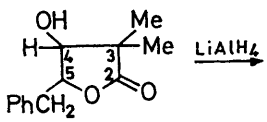
$EtMeCH\cdot CH(OH)\cdot CO_2H$

(III)

$PhCH_2\cdot CH(OH)\cdot CH(OH)\cdot CMe_2\cdot CO_2H$

(IV)

hydride gave the triol (V), which was oxidized by periodate to phenylacetaldehyde (VI) and 3-hydroxy-2,2-dimethylpropionaldehyde (VII), isolated as a dimer.[2] The activity was found only in the phenylacetaldehyde, as predicted from the original position of the ^{14}C in phenylalanine.



(IVa)

$\xrightarrow{LiAlH_4}$ $PhCH_2\cdot CH(OH)\cdot CH(OH)\cdot CMe_2\cdot CH_2\cdot OH$

(V)

$\xrightarrow{HIO_4}$ $PhCH_2\cdot CHO$ + $OHC\cdot CMe_2\cdot CH_2\cdot OH$

(VI) (VII)

The experiment was repeated with [1-^{14}C]phenylalanine, in order to ascertain whether C-4 in structure (IVa) originated from the carbonyl group. The resulting neoantimycin was indeed active (Table 1), the activity remained in the lactone (IVa), but reduction and oxidation as before gave inactive phenylacetaldehyde; all the activity was found in the hydroxy-aldehyde (VII).

The foregoing experiments indicate that phenylalanine (or a biochemical equivalent such as phenylpyruvic acid) is a biological precursor of neoantimycin, and that the skeleton of phenylalanine is totally incorporated into fragment (IV).

The origin of the $-CMe_2\cdot CO_2H$ group of fragment (IV) was investigated by use of similar techniques. The presence of a geminal dimethyl group suggested that methionine could be a precursor.

Incubation of cultures of *S. orinoci* with [*Me*-^{14}C]-methionine gave active neoantimycin (Table 1); the activity remained in the lactone fragment (IVa) and was retained in compound (VII).

If the incorporation of methionine accounted for only one of the two methyl groups of fragment (IV), the three carbon atoms whose origin had not yet been determined could have originated from a propionate unit. If, however, both the methyl groups came from methionine, only a two-carbon unit (*e.g.* acetate) was necessary to complete the fragment. Use of procedures similar to those already described indicated that [2,3-^{3}H]propionate was incorporated into structure (VII).

On the basis of the experiments summarized in Tables 1 and 2, we conclude that the acid (IV) originates from the condensation of a molecule of phenylalanine and a molecule of propionic acid, one methyl group of the geminal dimethyl system coming from methionine. The biosynthesis of fragment (IV) appears to be the first example of *in vivo* condensation of a shikimate-derived C_6–C_3 fragment with a propionic acid residue.

EXPERIMENTAL

The labelled precursors were purchased from the Radiochemical Centre, Amersham, Bucks. Radioactivities were measured by the liquid scintillation method (Tricarb 3375 spectrometer). Absolute values were determined by the channel ratio method.

[^{14}C]*Neoantimycin.*—*S. orinoci* [1] was grown in 300-ml Erlenmeyer flasks each containing a medium (60 ml) consisting of glucose, distillers' solubles, and inorganic salts in tap water. The flasks were kept for 120 h at 28 °C on a rotary shaker. The labelled precursors were added after incubation for 48 h (added radioactivity was *ca.* 20—25 μCi per flask for ^{14}C-labelled precursors and 1·43 mCi per flask for [2,3-^{3}H]propionate). At harvest the mycelium was extracted twice with methanol and finally with acetone, and the combined extracts were concentrated under reduced pressure, diluted with water, and re-extracted with ethyl acetate. The filtered broth was extracted with ethyl acetate and the extracts were concentrated. In the [2-^{14}C]phenylalanine experiment purification was performed by column chromatography [1] followed by addition of carrier neoantimycin and repeated crystallization from benzene–hexane to constant specific activity. In the other experiments the extracted material was chromatographed on thin-layer silica gel plates, with ethyl acetate as developing solvent. The fluorescent band of neoantimycin was scraped off and the product eluted with ethyl acetate. The chromatographic purification was repeated to obtain radiochemical purity (single radioactive peak on t.l.c.). The yield of neoantimycin varied between 100 and 200 μg per ml of culture.

TABLE 1

Incorporation of radioactivity into neoantimycin

Precursor	Added radioactivity (μCi)	Neoantimycin Yield (%)	Specific activity (nCi mg^{-1})	Incorporation (%)
[2-^{14}C]Phenylalanine	40	62·5 [a]	4·12	0·65
[1-^{14}C]Phenylalanine	40	24·7 [b]	36·0	2·22
[*Me*-^{14}C]Methionine	50	11·1 [b]	27·4	0·61
[2,3-^{3}H]Propionate	10,000	67·0 [b]	2160	1·45

[a] Carrier neoantimycin (80 mg) added. [b] No carrier added.

Neoantimycin Methyl Ether.—The samples of the labelled neoantimycin in ether–methanol were methylated with ethereal diazomethane. The product was generally recrystallized from ethyl acetate–hexane to yield pale yellow crystals, m.p. 107—108° (85% yield).[2] The samples were added to carrier neoantimycin methyl ether; the values of radioactivity obtained are reported in Table 2.

Alkaline Hydrolysis of Neoantimycin Methyl Ether.—Alkaline hydrolysis was performed as reported previously.[2] The sole labelled compound obtained, containing all the

radioactivity, was the γ-lactone (IVa), which was isolated in each experiment from the neutral fraction. The samples of lactone were purified by fractional crystallization; relative radioactivities are reported in Table 2.

The mixture of the remaining acids (I) (as methyl ether), (II), and (III) only contained traces of radioactivity.

(silica gel plates; 30% ethyl acetate in hexane) of the product showed two spots and the material was purified by preparative t.l.c. Qualitative evaluation of the radioactivity was performed with a radiochromatogram scanner. The compound of higher R_F value corresponded to phenylacetaldehyde (VI), which showed high radioactivity only

TABLE 2

Incorporation of:	*O*-Methylneoantimycin (nCi mg^{-1})	Lactone (IVa) (nCi mg^{-1}) [a]	Aldehyde (VI)	Hydroxy-aldehyde (VII)
[2-^{14}C]Phenylalanine	5·79	16·74 (18·77)	Active	Inactive
[1-^{14}C]Phenylalanine	2·92	8·61 (9·46)	Inactive	Active
[*Me*-^{14}C]Methionine	2·00	6·54 (6·48)	Inactive	Active
[2,3-^{3}H]Propionate	220	711 (713)	Inactive	Active

[a] Calculated values in parentheses.

Reduction of γ-Lactone (IVa) *by Lithium Aluminium Hydride.*—The labelled lactone (IVa) (*ca.* 150 mg) in ether was reduced to the triol (V) by refluxing for 4 h with ethereal lithium aluminium hydride. The crude triol was obtained as a pale yellow oil.

Periodate Oxidation of the Triol (V).—The oxidation was carried out as described for the inactive triol.[2] T.l.c.

in the experiment with [2-^{14}C]phenylalanine. The compound of lower R_F value corresponded to 3-hydroxy-2,2-dimethylpropionaldehyde (VII) and showed high radioactivity only in the experiments with [1-^{14}C]phenylalanine, [*Me*-^{14}C]methionine, and [2,3-^{3}H]propionate (see Table 2).

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