269. Biosynthesis of Cytochalasans. Part 5. The Incorporation of Deoxaphomin into Cytochalasin B (Phomin) [1])

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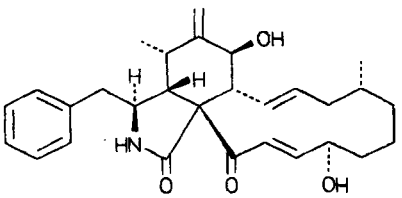
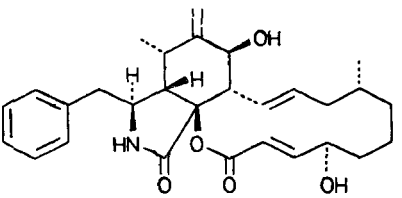
(7. X. 75)

Summary. Radioactive deoxaphomin (**1**) which was obtained by feeding [4'-^{3}H, U-^{14}C]-L-phenylalanine to cultures of *Phoma sp.* (S 298) was shown to be well incorporated into cytochalasin B (phomin) (**2**). The results demonstrate that **1** is an immediate biogenetic precursor of **2**.

1. Introduction. – During the biosynthesis of cytochalasin B (phomin) (**2**) by *Phoma sp.* (S 298) one unit of phenylalanine is attached to an acetate-malonate derived C_{18}-polyketide which contains two C_1-units originating from the methyl group of methionine [2] [3]. Recent isolation of deoxaphomin (**1**) [4], proxiphomin, and protophomin [5] suggested that a carbocyclic [13]cytochalasan could generate the macrolide system of cytochalasin B (**2**) by an enzymatic *Baeyer-Villiger* type oxygen insertion between C(9) and C(23) [6] [7]. A similar microbiological oxygen insertion reaction has recently been observed in the ansamycin series, namely in the transformation of rifamycin W to rifamycin B [8]. Such oxidations also occur during microbial formation of testololactone from androst-4-ene-3,17-dione or of testosterone acetate from progesterone [9]. In the case of cytochalasin B (**2**), deoxaphomin (**1**) appeared a particularly likely precursor because it possesses the same carbon skeleton and absolute configuration as **2** [4].



1 Deoxaphomin



2 Cytochalasin B (Phomin)

2. Incorporation Experiments. – Before testing the above hypothesis radioactive deoxaphomin (**1**) was prepared by administration of [4'-^{3}H, U-^{14}C]-L-phenylalanine to cultures of *Phoma sp.* (S 298). This precursor was chosen because it is well incorporated into cytochalasans in a specific manner, and the radioactivity of the product is easily localized by degradation to benzoic acid (**3**) [2]. In addition a constant ^{3}H/^{14}C ratio gives evidence of intact incorporation. After fermentation was complete, deoxaphomin (**1**) and cytochalasin B (**2**) were separated by careful fractional crystallization and preparative thin layer chromatography. The purity of isolated

[1]) Part 4, see [1].

radioactive deoxaphomin (**1**) was checked by dilution with inactive cytochalasin B (**2**), repetition of the separation process, and measurement of the radioactivity of both substances. This procedure was necessary because deoxaphomin (**1**) is amorphous. In this way it was shown that active deoxaphomin (**1**) contained no more than 1.4% of active cytochalasin B (**2**).

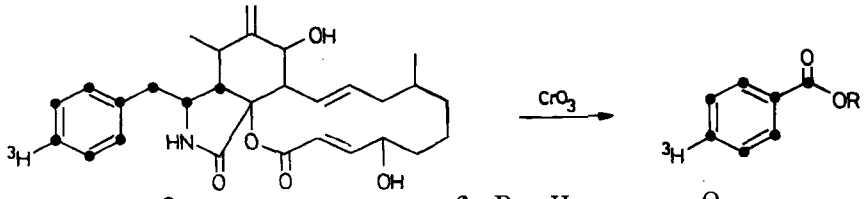
In two independent experiments different quantities of radioactive deoxaphomin (**1**) were added to growing cultures of *Phoma sp.* (S 298) after production of cytochalasin B (**2**) had started. This method avoided degradation of the precursor prior to incorporation. The resulting cytochalasin B (**2**) was isolated as before and degraded by a modified *Kuhn-Roth* oxidation [2] to benzoic acid (**3**) which was transformed into the *p*-bromo-phenacyl ester **4**. Results of the radioactivity determinations are summarized in the Table.

Table. *Incorporation Experiments*[a])

Precursor	*Activity*		$^3H/^{14}C$
	Total (mCi)	*Specific* (mCi/mmol)	Activity Ratio
[4′-^{3}H, U-^{14}C]-L-Phenylalanine	^3H: 5 ^{14}C: 1	$1.05 \cdot 10^4$ $5.13 \cdot 10^2$	4.8
[^{3}H,^{14}C]-Deoxaphomin (**1**)			
1. Experiment	^3H: $1.10 \cdot 10^{-3}$ ^{14}C: $2.38 \cdot 10^{-4}$	0.27 0.06	4.6
2. Experiment	^3H: $2.20 \cdot 10^{-3}$ ^{14}C: $4.79 \cdot 10^{-4}$	0.27 0.06	4.6

Product	*Activity* (dpm/mg)	(dpm/mmol)	*Incorp. Rate* Absolute	Specific	$^3H/^{14}C$ Activity Ratio
Deoxaphomin (**1**)	^3H: $1.31 \cdot 10^6$ ^{14}C: $2.84 \cdot 10^5$	$6.06 \cdot 10^8$ $1.31 \cdot 10^8$	– –	$2.60 \cdot 10^{-5}$ $1.15 \cdot 10^{-4}$	4.6
Cytochalasin B (**2**)					
1. Experiment	^3H: $27.60 \cdot 10^2$ ^{14}C: $5.73 \cdot 10^2$	$1.32 \cdot 10^6$ $2.74 \cdot 10^5$	8.8%	$2.12 \cdot 10^{-3}$	4.8
2. Experiment	^3H: $49.90 \cdot 10^2$ ^{14}C: $10.50 \cdot 10^2$	$2.39 \cdot 10^6$ $5.02 \cdot 10^5$	7.0%	$3.88 \cdot 10^{-3}$	4.8
p-Bromphenacyl-benzoate (**4**)[b])	^3H: $41.79 \cdot 10^2$ ^{14}C: $7.22 \cdot 10^2$	$1.33 \cdot 10^6$ $2.30 \cdot 10^5$	–	–	5.8[c])

[a]) The activities given are minimum values as determined by dilution experiments.
[b]) Obtained from the 1. Experiment.
[c]) Calculated $^3H/^{14}C$ ratio is 6.1.



2 **3** R = H **4** R = *p* · Br—C_6H_4—C(=O)—CH_2

3. Discussion. – Comparison of the $^3H/^{14}C$ ratios of deoxaphomin (**1**) and L-phenylalanine demonstrates that the amino acid is incorporated into the [13]cytochalasan **1** with retention of the carbon skeleton, a result observed earlier for the biosynthesis of cytochalasin B (**2**) [2]. The high rate of incorporation and the unchanged $^3H/^{14}C$ ratio in cytochalasin B (**2**) produced in the second experiment shows that a significant incorporation of deoxaphomin (**1**) has been achieved. As expected, the $^3H/^{14}C$ ratio of *p*-bromo-phenacyl benzoate (**4**) corresponds to 7/9 of the ^{14}C-activity and to the total ^3H-activity of **2**. On the basis of these results we conclude that deoxaphomin (**1**) is a direct precursor of cytochalasin B (**2**) in *Phoma sp.* (S 298). An analogous *Baeyer-Villiger* type oxidation can be postulated for the biosynthesis of cytochalasin E [10] in *Aspergillus clavatus* or *Rosellinia necatrix*, whereby two oxygen atoms are inserted into a carbocyclic [11]cytochalasan to form the carbonic ester.

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Experimental Part.

1. *General methods.* Melting points were obtained on a *Kofler* block and are corrected. Samples for radioactivity measurements were dried at least 2 h at 23°/0.01 Torr. Silica gel 0.05–0.2 mm from *E. Merck AG.*, Darmstadt, was employed for column chromatography. Thin-layer chromatography (TLC.) was carried out on silica gel PF 254 (*Merck*) and on silica gel G (*Merck*).

We are indebted to Mr. *H. Galliker* and Mr. *G. Marbach, Sandoz AG.*, Basel, for the radioactivity determinations which were carried out directly on a *Packard* Tri-Carb Model 3375 liquid scintillation spectrometer.

2. *Isolation of radioactive deoxaphomin* (**1**). Fermentation of *Phoma sp.* S 298 and isolation of the resulting deoxaphomin (**1**) was accomplished in the manner previously described [4]. The culture medium (10 l) was treated with radioactive [4′-^{3}H, U-^{14}C]-L-phenylalanine before inoculation. After 12 days at 18° the culture filtrate and micelium were worked-up and the extracts chromatographed on silica gel to produce: 29 mg of pure deoxaphomin (**1**), identical with authentic material.

3. *Incorporation of radioactive deoxaphomin* (**1**) *and isolation of cytochalasin B* (*Phomin*) (**2**). a) A solution of 1.89 mg ($4.08 \cdot 10^{-3}$ mmol) of deoxaphomin (**1**) in 10 ml of H_2O/CH_3OH 4:1 was injected into a 4 day old growing culture (1 l) of *Phoma sp.* S 298 at several points under the micelium. After 8 days 80 mg of pure **2** were isolated as described before [2]. b) The same method was used to produce 70 mg of pure **2** from 3.78 mg ($8.16 \cdot 10^{-3}$ mmol) of **1**.

4. Kuhn-Roth *degradation of cytochalasin B* (*Phomin*) (**2**). A 35 mg (0.073 mmol) portion of **2** was heated with 6 ml of *Kuhn-Roth* reagent (10 g CrO_3, 90 ml H_2O, 15 ml H_2SO_4) in a sealed glass tube for 2 h at 130° (shaking autoclave). The resulting acetic acid and benzoic acid (**3**) were steam distilled until 400 ml had been collected. The distillate was titrated with 0.01 N NaOH against phenolphthaleine and evaporated *in vacuo*. The sodium salts were refluxed with 100 mg (0.36 mmol) of *p*-bromo-phenacyl bromide for 2.5 h in 1.5 ml H_2O and 3 ml CH_3OH. Evaporation of the solvent and chromatography of the residue (130 mg) on silica gel (benzene/methanol 97:3, benzene) yielded 12 mg (0.037 mmol, 50%) of *p*-bromo-phenacyl benzoate (**4**), identical with authentic material (TLC., mixed m.p.).

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270. A New Synthesis of β-Lactams

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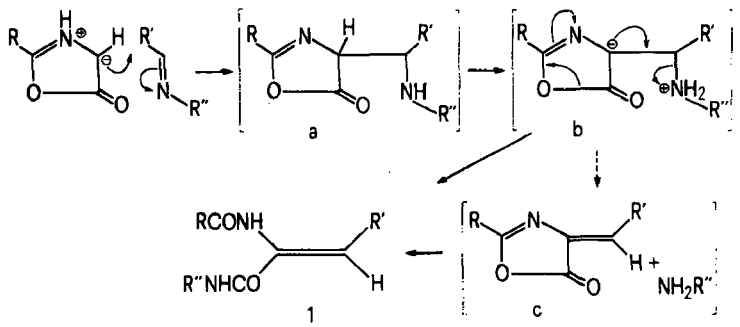
(23. IX. 75)

Zusammenfassung. Es wird eine neue Synthese von β-Lactamen durch Umsatz von 4-Alkyl-azlactonen mit acyclischen Iminen beschrieben. Mit einem cyclischen Imin wird dagegen ein Imidazolin-Derivat erhalten.

The reaction of oxazolin-5-ones with different imines is reported in the literature [1–3]. In all cases the products obtained are derived from an initial nucleophilic attack of oxazolone on the imine (*Scheme 1*), and are of type **1**.

Scheme 1



During our work on β-lactam antibiotics, we got interested in the above scheme as we have visualized the possibility of obtaining β-lactams by substituting one of the hydrogen atoms at C(4) of oxazolone by an alkyl group. The intermediate **d** (*Scheme 2*) generated by the initial attack of the oxazolone on an imine, can now lead to an azetidinone **2** and/or an imidazoline derivative **3** as shown in *Scheme 2*. The imidazoline derivative is of interest because of its close relationship to penillic acid (**4a**),

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