Isolation and Structure of Phomin

Phomin is a new antibiotic which we have isolated from cultures of a *Phoma* species (strain S 298) (*Fungi imperfecti*)[1]. It exhibits cytostatic activity in vitro. Multiplication of HeLa cells in vitro is prevented completely by concentrations of 3–10 μg/ml. Furthermore, migration of chicken leucocytes is 80% inhibited by a concentration of phomin of 2.5 μg/ml[2].

Phomin, $C_{29}H_{37}NO_5$[3], is a neutral lipophilic substance crystallizing in colourless needles, m.p. 218–220°; $[\alpha]_D^{25}$ + 83° ± 2° (methanol). On the basis of the following evidence we assign structure **1** to phomin. Its UV-spectrum (ethanol) exhibits intense absorption maxima at 213 (4.42) and 219 (4.32) nm (log ε) with inflections at 258 (2.69), 264 (2.48) and 267 (2.32) nm (log ε), which indicate the presence of several isolated double bonds and of an aromatic ring. The IR-spectrum (nujol and KBr) shows frequencies at 3510 (OH); 3370, 3220 and 3140 (NH); 3090, 3060 and 3025 (=CH– and $=CH_2$); 1715 and 1690 (2C=O); 958 (–CH=CH-*trans*); 988 (possibly –HC=CH-*trans*); 1814, 1638 and 901 (R'R''C=CH_2); 1600, 1580, 1490, 760 and 690 (monosubstituted benzene ring); 1370 ($-CH_3$); 1270–1250 (C–O, ester) cm^{-1}. Phomin (**1**) forms a di-*O*-acetyl derivative **2** ($C_{33}H_{41}NO_7$, amorphous [+ 98 Al])[4]. In the IR-spectrum the NH band is still present at 3410 cm^{-1} but the hydroxyl frequencies have disappeared. When an ethanolic solution of phomin (**1**) is hydrogenated in the presence of Pd as a catalyst, hexahydro-phomin (**5**) ($C_{29}H_{43}NO_5$, m.p. 193–196° [+ 6 Al]) is obtained. Its UV-spectrum exhibits 6 absorption maxima in the region of 242.5–268 nm with the main peak being at 258.5 nm (log ε = 2.37). The fine structure is characteristic for a benzene ring. Acetylation of **5** yields di-*O*-acetyl-hexahydro-phomin (**6**) ($C_{33}H_{47}NO_7$, amorphous [+ 12 Al]). Further hydrogenation of **5** can be achieved with Pt in acetic acid leading to dodecahydro-phomin (**7**) ($C_{29}H_{49}NO_5$, m.p. 185–188° [+ 13 Al]); di-*O*-acetyl derivative **8** ($C_{33}H_{53}NO_7$, amorphous [0 Al]). Under these conditions dodecahydro-phomin (**7**) is also obtained from phomin (**1**). Compounds **7** and **8** are transparent in the UV-region. It is concluded from these observations that phomin (**1**) contains 2 hydroxyl groups. They are secondary since the NMR-spectrum[5] of **7** in d-DMSO solution shows 2 doublets at 4.28 and 4.68 ppm[6] which disappear after treatment with D_2O.

A NH group is present in phomin (**1**) appearing as singlet at 8.05 ppm in the NMR-spectrum. This proton is readily exchanged by deuterium. The NH group participates in a γ-lactam function, as is indicated by the presence of the typical amide-I band at 1715 cm^{-1} and the absence of an amide-II band in the IR-spectrum[7]. The NMR-spectrum of phomin (**1**) reveals 2 secondary methyl groups (doublets at 0.63 ppm, J = 6 c/sec, and at 0.82 ppm, J = 6 c/sec). By the UV-, IR- and NMR-spectra (multiplet of 5 protons at ca. 7.14 ppm) and the

[1] The fermentations were carried out by DR. CH. STOLL and Dr. E. HÄRRI, Sandoz AG, Basel, Switzerland. We are indebted to them for providing the culture broths

[2] We should like to thank Dr. H. STÄHELIN, Sandoz AG, Basel, very much for these tissue culture tests.

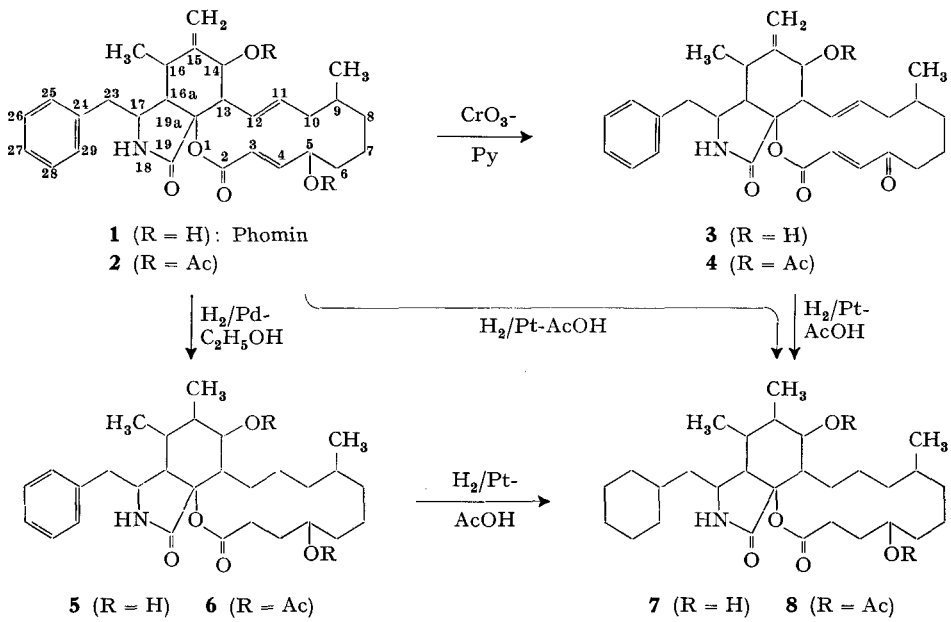
[3] Derived from the elemental analysis and the mass spectrum. We wish to express our gratitude to Dr. W. VETTER, F. Hoffmann-La Roche & Co. AG, Basel, for the measurement and the discussion of the mass spectra. They were determined by an AEI Ltd. MS-9 Mass Spectrometer equipped with a direct inlet system.

[4] The numbers in angular brackets denote the values of the specific optical rotations for Na-light. Abbreviations: Al = ethanol, Chf = chloroform.

[5] The NMR-spectra were measured by a Varian Spectrometer A-60 (60 MHz) in our Institute (K. AEGERTER) or by a Varian Spectrometer HR-100 (100 MHz) of Varian AG, Zürich (Dr. U. SCHEIDEGGER). Chemical shifts in δ-values with $Si(CH_4)_4$ (TMS) as internal standard (δ = 0). Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet. The numbers are the spin-spin-coupling constants J in c/sec.

[6] Cf. O. L. CHAPMAN and R. W. KING, J. Am. chem. Soc. *86*, 1256 (1964); J. G. TRAYNHAM and G. A. KNESEL, J. Am. chem. Soc. *87*, 4220 (1965).

[7] R. MECKE JR. and R. MECKE SEN., Ber. dt. chem. Ges. *89*, 343 (1956).



1 (R = H): Phomin
2 (R = Ac)

3 (R = H)
4 (R = Ac)

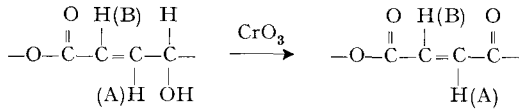
5 (R = H) **6** (R = Ac)

7 (R = H) **8** (R = Ac)

Ac = CH_3CO–: Py = pyridine

hydrogenation experiments, the presence of a mono-substituted benzene ring in phomin (**1**) has been demonstrated. This benzene ring is incorporated in a benzyl group as evidenced by mass spectral data. Both phomin (**1**) (M^+ at mass 479) and hexahydro-phomin (M^+ at mass 485) show the 'base peak' at m/e 91 which corresponds to the fragment $C_7H_7^+$ (benzylium ion, which isomerizes to give the more stable tropylium ion). In dodecahydro-phomin (**7**) this fragment is absent. In **1** and **5** the M-91 peaks and in **7** the M-97 peak are also well recognized. In the NMR-spectra the chemical shift of the 2 benzyl protons at C-23 is as expected (e.g. in di-*O*-acetyl-phomin (**2**) doublet at 2.80 ppm, J = 6c/sec).

The fourth double bond of phomin is present as vinylidene group, as demonstrated by the formation of CH_2O and the γ-lactam II (**19**) upon treatment with O_3, a reaction which is discussed later. Of the 2 remaining *trans*-substituted double bonds one is conjugated with a carbonyl group (ν[C=O]= 1690 cm^{-1}) which is shifted to 1725–1730 cm^{-1} in the hydrogenation products **5** and **7**. Actually phomin (**1**) is an α,β-unsaturated ester. The environment of this functional group was established in the following manner. Treatment of **1** with CrO_3-pyridine[8,9] yields dehydro-phomin (**3**) ($C_{29}H_{35}NO_5$, m.p. 185–187° [+ 92 Al])[10]. It forms a mono-*O*-acetyl derivative **4** ($C_{31}H_{37}NO_6$, amorphous [+ 18 Chf]). The IR-spectrum of **3** (ν[C=O] = 1618 cm^{-1}) and the UV-spectrum (λ_{max} = 227 nm; log ε = 4.09) indicate the presence of an α,β-unsaturated ketone group which originates from an allylic hydroxyl function:

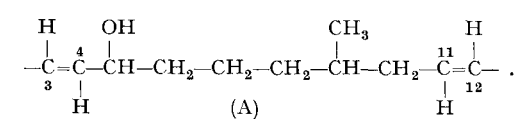


The NMR-spectrum confirms this interpretation. In phomin (**1**) the vinylic proton A is split into a double doublet (6.77 ppm). Proton B appears as a doublet at 5.77 ppm (J = 16 c/sec). In dehydro-phomin (**3**) the pattern of the AB-system of the *trans* double bond is simplified. The signals of both protons A and B are now doublets appearing at 7.42 and 6.43 ppm (J = 16 c/sec).

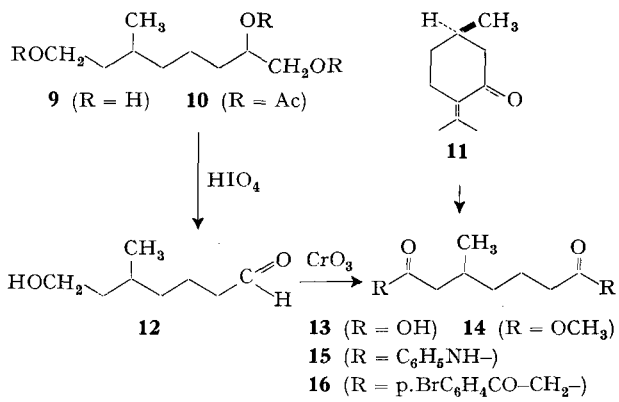
Further insight into the structure of phomin (**1**) was gained by the ozonolysis of di-*O*-acetyl-phomin (**2**) and subsequent reductive cleavage of the ozonides with $NaBH_4$ and reacetylation. 4 products were obtained:

(1) Formaldehyde, isolated as dimedone derivative.

(2) 1,7,8-Triacetoxy-3-methyl-octane (**10**) ($C_{15}H_{26}O_6$, oil b.p. 120°/0.01 Torr [+ 2 Chf]). Deacetylation with $LiAlH_4$ gave the triol **9**. Cleavage of **9** with HIO_4 yielded formaldehyde and the hydroxy-aldehyde **12** ($C_8H_{12}O_2$, oil [+ 2 Chf]). Oxidation of the latter by CrO_3-H_2SO_4 in acetone[11] yielded the known R-(+)-3-methyl-pimelic acid (**13**) ($C_8H_{14}O_4$, oil [+ 7.9 Al])[12] (dimethyl ester **14**: $C_{10}H_{18}O_4$, oil b.p. 239° [+ 6 Chf]; dianilide: $C_{20}H_{24}N_2O_2$, m.p. 161–162° [+ 15 Al]; di-*p*-bromophenacyl ester: $C_{24}H_{24}O_6Br_2$, m.p. 109–111° [+ 7 Chf]). The absolute configuration of (+)-3-methyl-pimelic acid has been established by its interconnection with (+)-pulegone (*p*-menth-4(8)-en-3-one) **11**[13]. Due to the formation of the triol **9**, partial structure A can be deduced for phomin (**1**):

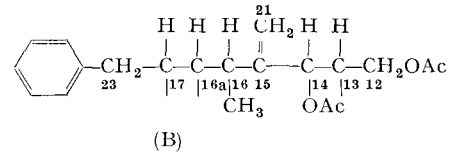


(3) γ-Lactam I **17** ($C_{18}H_{23}NO_4$, m.p. 187–188.5°, [+ 88 Al])[14]; tri-*O*-acetyl-derivative **18** ($C_{24}H_{29}NO_7$, amorphous [+ 49 Al]).



(4) γ-Lactam II (**19**) ($C_{17}H_{23}NO_5$, amorphous [– 15 Al])[14]; tetra-*O*-acetyl derivative **20** ($C_{15}H_{31}NO_9$, m.p. 135–138°, [– Al]). The γ-lactam I (**17**) is transformed to the γ-lactam II (**19**) by further treatment with O_3, etc. The latter compound consumes 1 mole of HIO_4, whereas **17** is stable to HIO_4. Therefore the γ-lactam I (**17**) and hence also phomin (**1**) contain a $H_2C{=}C{-}\overset{|}{C}{-}OH$ group.

Spin-spin decoupling experiments by double resonance experiments at 100 MHz[15] with the tri-*O*-acetyl-γ-lactam I (**18**) by irradiation of the C-12 (4.86 ppm, dd/12/4), C-13 (2.87 ppm, ddd/4/6/11), C-14 (5.73, d/11), C-16 (3.13, dq/5/6.5), C-17 (3.27, dt/5/7) and C-23 (2.88, d/7) protons enabled us to establish the following sequence B:



(B)

Long-range coupling of the protons at C-14 and C-16 is observed. It also gives rise to the slight splitting of the 2 vinylic protons at C-21 (2 triplets at 5.12 and 5.22 ppm, J = 1.5 c/sec). The signal at 2.73 ppm (dd/5/5) is assigned to the proton at C-16a and the doublet (J = 7 c/sec) at 2.88 ppm to the 2 protons at C-23. The attachment of the nitrogen atom of the amide group to the carbon atom which carries the benzyl group (C-17) follows from the mass spectral data. Whereas in phomin (**1**) the 'base peak' is the benzylium or tropylium ion (m/e 91) the 'base peak' in the secondary amine **23** (see below) is the fragment M-91 (m/e 380) (type-A_4 fragmentation vs.

[8] G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, J. Am. chem. Soc. *75*, 425 (1953).

[9] Treatment of phomin with CrO_3 in acetic acid or with CrO_3-H_2SO_4 in acetone[11] gave very complex mixtures of products which could not be separated.

[10] Dehydro-phomin (**3**) was isolated occasionally also from the culture broth. It is not clear whether this compound is a real metabolite or an artefact.

[11] K. Bowden, I. M. Heilbron, E. H. R. Jones, and B. C. L. Weedon, J. chem. Soc. *1946*, 39.

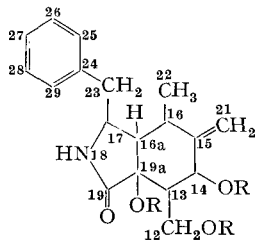
[12] M. Mousseron and J. Jullien, Bull. Soc. chim. France *1947*, 605.

[13] Cf. A. J. Birch, Rep. Prog. Chem. *47*, 190 (1950).

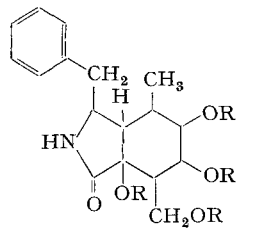
[14] Isolated after hydrolysis of the crude mixture of the acetylated ozonolysis products by K_2CO_3 in methanol.

[15] We are indebted very much to Dr. U. Scheidegger, Varian AG, Zürich, for carrying out and discussing the double resonance experiments.

type-B fragmentation[16]). Attachment of the remaining ≥C–OH group to the amide, extended sequence B, leads to structure **17** for the γ-lactam I. This formula allows 32 possible stereoisomers, of which 28 can be ruled out on the basis of the NMR-data using the KARPLUS equation[17]. The remaining 4 stereoisomers possess the following configuration if the β-configuration[18] is assigned to H-atom at C-16a: 13α, 14β, 16α, 17α, 19aα; 13α, 14β, 16α, 17α, 19aβ; 13β, 14α, 16α, 17β, 19aβ; and 13α, 14β, 16β, 17β, 19aβ. One isomer is derived from *trans*- and the other 3 from *cis*-octahydro-isoindol. For the bicyclic system only one conformation is possible.

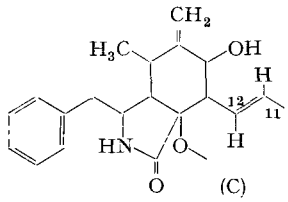


17 (R = H): γ-Lactam I
18 (R = Ac)



19 (R = H): γ-Lactam II
20 (R = Ac)

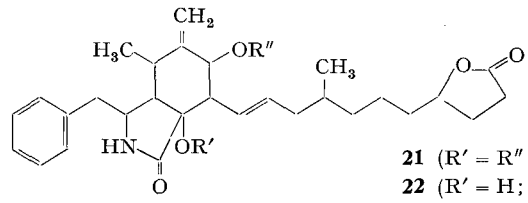
The primary and tertiary hydroxyl groups of the γ-lactams **17** and **18** are not present in phomin (**1**). They have been generated in the course of the ozonolysis of the 2 *trans*-disubstituted olefinic double bonds of di-*O*-acetyl-phomin (**2**). Thus partial structure C can be written for phomin (**1**) which is to be connected to the partial structure A deduced earlier.



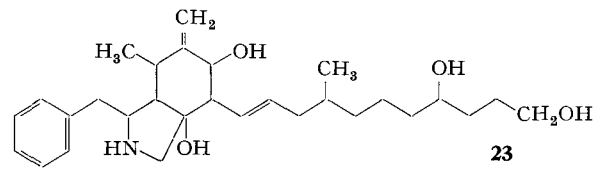
(C)

The decision of choosing between the 2 possibilities of combining structure A with structure C was achieved by spin-spin decoupling experiments[15] on di-*O*-acetyl-phomin (**2**). The protons at C-3 (5.78 ppm, d/16), C-4 (6.91 ppm, dd/16/5), C-5 (5.4 ppm, m), C-11 (5.4 ppm, m) C-12 (5.85, dd/16/9) and C-13 (3.56, dd/9/11) were irradiated. The evaluation of these NMR results leads to structure **1** for phomin. The glycolic acid formed after the hydrolysis of the ozonolysis products was not isolated.

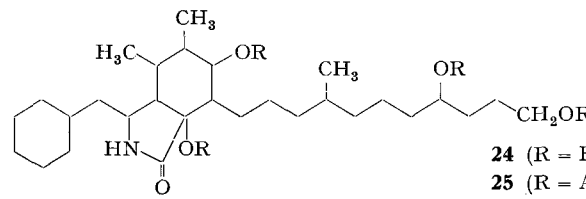
Further proof for the lactone and lactam functions arises from the reduction of phomin (**1**) and dodecahydro-phomin (**7**) with $LiAlH_4$. On treatment of **1** with 1.5 moles of $LiAlH_4$ in tetrahydrofuran for 1.5 h the γ-lactone **21** ($C_{29}H_{39}NO_5$, m.p. 192–194° [+ 99 Al]) is obtained. The molecular formula is confirmed by the mass spectrum (M^+ at m/e 481). In the IR-spectrum the lactam group is unchanged, but an additional new carbonyl absorption appears at 1764 cm^{-1}. The formation of the γ-lactone **21** can be explained by assuming that first the double bond of the α,β-unsaturated ester is hydrogenated (1,2-addition) and then a *trans*-esterification takes place. The NMR-spectrum is in full agreement with structure **21**. Compound **21** forms the mono-*O*-acetyl derivative **22** ($C_{31}H_{41}NO_6$, m.p. 194–196° [+ 38 Chf]). Treatment of phomin (**1**) with an excess of $LiAlH_4$ in tetrahydrofuran for 22 h yields the secondary amine **23** ($C_{29}H_{45}NO_4$, m.p. 136–138°). No carbonyl frequency can be observed in the IR-spectrum. The mass spectrum shows the M^+ peak (m/e 471) as expected. On refluxing di-*O*-acetyl-dodecahydro-phomin (**8**) with $LiAlH_4$ in ether a selective reduction of the ester bond takes place, yielding the tetra) hydroxy-lactam **24** ($C_{29}H_{53}NO_5$, amorphous [– 16 Al]-which gives a tetra-*O*-acetyl derivative **25** ($C_{37}H_{61}NO_9$, amorphous [– 1 Chf]).



21 (R′ = R″ = H)
22 (R′ = H; R″ = Ac)



23



24 (R = H)
25 (R = Ac)

Phomin represents a novel type of macrolide antibiotic, the large lactone ring being fused to a highly substituted octahydro-isoindol system.

The details of these investigations[19] are to be published in Helv. chim. Acta.

Zusammenfassung. Aus Kulturen eines *Phoma spec.* (Stamm S 298) (*Fungi imperfecti*) wurde das neue cytostatisch wirksame Antibioticum Phomin isoliert. Auf Grund seiner chemischen und physikalischen Eigenschaften wird ihm die Strukturformel **1** erteilt.

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Institut für Organische Chemie der Universität Basel, Basel (Switzerland), September 10, 1966.

[16] Cf. K. BIEMANN, *Mass Spectrometry* (McGraw-Hill Book Co., New York 1962), pp. 84, 87.

[17] M. KARPLUS, J. chem. Phys. *30*, 11 (1959); H. CONROY, Adv. org. Chem. *2*, 311 (1960); M. KARPLUS, J. Am. chem. Soc. *85*, 2870 (1963).

[18] This assignment and the adoption of the α,β-nomenclature are purely arbitrary and only used for brevity.

[19] They were supported by Sandoz AG, Basel, Switzerland and by a grant of the 'Schweizerische Nationalfonds zur Förderung der Wissenschaften' (Projects Nos. 2627, 3524 and 3945).