12. Structure of Wortmin, a new Metabolite from *Penicillium wortmanni*

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(Dedicated to Prof. Dr. M. Viscontini for his 60th birthday)

(31. X. 72)

Summary. The structure of ester of 2-hydroxy-4-methoxy-6-methylbenzoic acid with 6-acetoxy-7-hydroxy-7-methyl-3-propenyl-3,4,5,6,7,8-hexahydro-1*H*-benzo[e]pyran-8-one (**1**) is assigned to wortmin, a new member of the sclerotiorin group of metabolites, produced by *Penicillium wortmanni* Klöcker.

Penicillium wortmanni Klöcker is a source of new interesting metabolites, which have received much attention recently. The antibiotic wortmannin [1], and the pigments skyrin [2], rugulosin [2] and flavomannin [3] have been isolated from different strains of the mould. We report here on the structure of a new metabolite, wortmin, which is produced by the CBS strain of *P. wortmanni*. Besides the above substances, also ergosterol and mitorubrinol were isolated [4, 5].

Wortmin **1** was obtained from the ethyl acetate extract of the culture, and purified by chromatography. It appears as a faint yellow solid, m.p. 108°, $[\alpha]_D^{20} = +6.3°$ ($c = 0.19$, MeOH), of composition $C_{24}H_{28}O_8$ (M.W. = 444). The IR. spectrum shows three CO bands at 5.72, 5.92 and 6.05 μ, whereas the UV. spectrum is reminiscent of an *o*-hydroxybenzoic acid derivative (λ_{max} 265 (ε 16,800) and 305 nm (ε 5,500)). These features, together with the presence in the NMR. spectrum of **1** (see below) of an Aryl-Me, one OMe, two aromatic meta protons, and one chelated OH, suggested the presence of an orsellinic acid-type moiety.

Methanolysis of **1** with sodium methoxide afforded methyl 2-hydroxy-4-methoxy-6-methylbenzoate (**3**) and a mixture of two products, **2** and **5**. Treatment of this mixture with CH_2N_2 gave **3** and methyl 2,4-dimethoxy-6-methylbenzoate (**4**), thus making the isolation of **5** easier. The compound **5** has formula $C_{13}H_{18}O_4$ (m.w. = 238), and its IR. spectrum shows only one CO band at 6.00 μ; the UV. absorption at 239 nm (ε = 8,100). Comparison of the NMR. and mass spectra of **1** and **5** shows that in the methanolysis also an acetoxy group has been hydrolyzed. Confirmation of this result was obtained by acid hydrolysis of **1** with dil. HCl, that gave deacetylwortmin (**6**) (m.w. = 402), which, by methanolysis, gave again **5**. Thus **1** must be a diester of the diol **5** with acetic acid and with 2-hydroxy-4-methoxy-6-methylbenzoic acid (**2**). Acetylation of **5** gave diacetyl-**5** (**7**) (m.w. = 322).

The analysis of the NMR. spectra of **1**, **4**, **5**, **6**, together with double and triple resonance experiments gave information on all the H atoms of the compound **1** (see

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tables 1 and 2). The Aryl-Me group, the OMe, the two aromatic meta protons and the chelated OH can be attributed to the aromatic ester moiety. The protons H_Y and H_Z are *trans* on a double bond: H_Y is adjacent to Me group, whereas H_Z is adjacent to H_M. The chemical shift of H_M indicates that it is close to an oxygen atom; it is also coupled with H_C and H_D, both on the same carbon. The chemical shift and the additional small couplings indicate that H_C and H_D are allylic (sequence A). The proton H_X is the X part of an ABX system. It can not be a vinylic proton, although at such a low field (6.09 δ) because it does not change by hydrogenation to tetrahydrowortmin (**9**). Moreover, H_X is shifted to 4.02 δ in the diol **5**, where both the ester groups have been hydrolyzed. In the monoester **6** it appears at 5.03 δ, whereas it is restored to 6.01 δ in the diacetate **7**. It follows that H_X must be on the carbon bearing the acetoxy group, and that the carbonyl (and not the aromatic ring, because of the same effect in the diacetate **7**) of the aromatic ester has a downfield effect of ca. 1 ppm on H_X. It is thus possible that the aromatic ester group is adjacent and *cis* to H_X. The protons H_A and H_B are on the same carbon, and have small couplings (homoallylic) with H_C, H_D and H_P and H_Q (sequences B and D).

Table 1. *Chemical shifts* (δ) *in* $CDCl_3$

	1	**6**	**5**	**9**
H_X	6.19	5.03	4.02	5.94
H_Y	5.78	5.77	5.77	a
H_Z	5.52	5.51	5.53	a
H_P	4.57	4.50	4.40	4.31
H_Q	4.30	4.28	4.32	3.44
H_M	4.09	4.05	3.98	3.32
H_A	2.58[b]	2.50[b]	2.45	2.15[a, b]
H_B	center	center	2.45	1.56[a, b]
H_C	2.30[b]	2.25[b]	2.31	~1.5[a, c]
H_D	center	center	2.17	~1.5[a, c]
Me—C=	1.71	1.73	1.72	0.92
Me—C—O	1.55	1.51	1.25	1.63
Me—CO	2.05	–	–	2.06
Me-Aryl	2.49	2.53	–	2.45
MeO	3.76	3.77	–	3.77
2 H arom.	6.25 6.28	6.23[d]	–	6.26 6.30
OH phenol.	10.05	11.20	–	11.09
OH alcoh.	–	3.1	3.8[e]	–
H_L	–	–	–	2.22[a, b]

a) overlapped by other protons on satd. fragments
b) from decoupling
c) not exactly localized
d) singlet
e) 2H

Table 2. *Coupling constants (Hz)**

	1	**5**	**9**
$J_{Y,Z}$	16.0	16.0	b)
$J_{Z,M}$	5.8	6.0	b)
$J_{Me,Y}$	6.0	6.0	d)
$^4J_{Me,Z}$	1.2	1.2	~0
$^5J_{Me,M}$	$\leqslant$ 1.1	$\leqslant$ 1	~0
$^4J_{Y,M}$	$\leqslant$ 0.7	$\leqslant$ 0.7	b)
$J_{P,Q}$	16.5	16.5	12.0
$^5J_{Q,A} \sim J_{Q,B}$	2.0	a)	0
$^5J_{Q,C} \sim J_{Q,D}$	1.0	a)	0
$^5J_{P,A} \sim J_{P,B}$	1.0	a)	0
$^5J_{P,C} \sim J_{P,D}$	1.0	a)	0
$J_{A,X}$	16.5 f)	16.0 f)	5.0 e)
$J_{B,X}$			11.5 e)
$J_{A,B}$	b)	b)	b)
$\lvert J_{MC}+J_{MD}\rvert$	12–14 e)	12–15 e)	e)
$J_{C,D}$	b)	18.0	b)
J_{meta}	2.5	–	2.5
$J_{P,L}$	–	–	4.0
$J_{Q,L}$	–	–	9.5

* All the coupling constants of **6** are identical to those of **5**.

a) The homoallylic couplings have been detected by decoupling, but they are not resolved, because $\delta_P \sim \delta_Q$

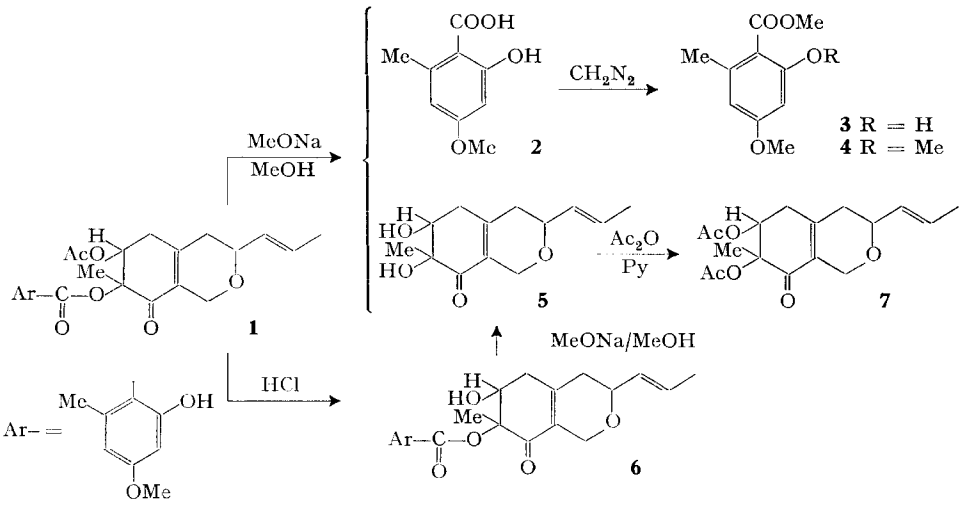
b) Not detected, because the protons are partially or completely overlapped by other signals

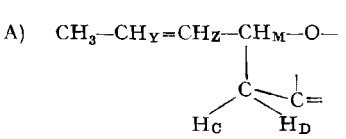
c) or *vice versa*

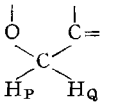
d) the Me signal is a degenerate triplet, due to the coupling with the close CH_2

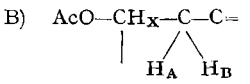
e) H_M is a broad signal with $W_{1/2}$ = 21–23 Hz

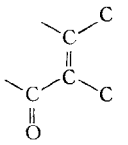
f) $\lvert J_{AX}+J_{BX}\rvert$.

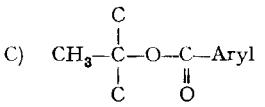


A) 

D) 

B) 

E) 

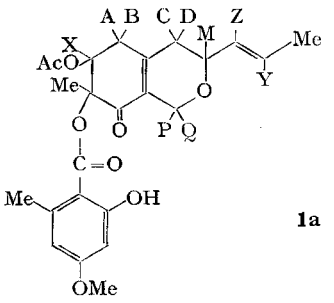
C) 

The singlet at 1.55 δ in **1** can be attributed to a Me $-\overset{|}{C}-O-$ group, and as it is shifted to higher field (1.25 δ) only in **5**, it is probable that it is on the carbon bearing the aromatic ester (sequence C). Also the protons H_P and H_Q are on the same carbon: their chemical shift, which is not affected by hydrolysis to **5** and **6**, suggests that they are near to an oxygen. These protons too show small couplings with H_A, H_B, H_C and H_D (sequence D). Actually, by hydrogenation to **9**, the ^5J disappear and all these six protons are shifted upfield.

The UV., IR. evidence, together with the existence of a number of small couplings in the NMR. spectrum indicate that wortmin must contain an unsubstituted double bond conjugated with a carbonyl group (sequence E).

Hydrogenation of **1** with $Pd/BaSO_4$ in ether gave dihydrowortmin (**8**) (m.w. = 446). No apparent change was observed in the UV. spectrum, whereas the disappearing of the protons H_Y and H_Z in the NMR., the upfield shift of the Me group from 1.71 δ to 0.92 δ (CH_3–CH_2–C$\lesssim$ degenerate triplet), and also the upfield shift of H_M to 3.4 δ confirm the sequence A.

All the evidence so far obtained indicated that the oxygen atom included in the sequences A and D should be the same, and also that most probably the sequences B and C were linked by a single bond. The existence of the homoallylic couplings between the protons H_P,H_Q and H_A,H_B suggested the formula **1a**.

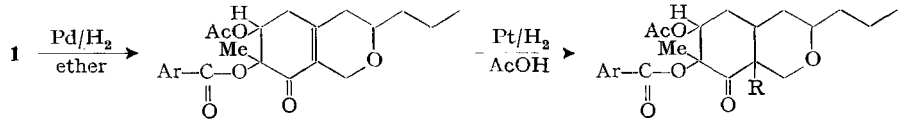


1a

Moreover, the structure **1a** is most probable on the basis of biogenetic arguments, owing to the close similarity with the members of the sclerotiorin (azaphilone) group

of metabolites [6], particularly with mitorubrin [4]. Wortmin appears as an interesting new example, with a low oxidation level.

Support for the structure **1** was also obtained by examining tetrahydrowortmin **9**, prepared by hydrogenation of **1** with Pt in AcOH.

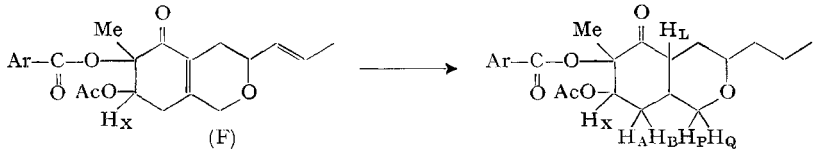


8 **9** R = H, **10** R = D

This compound ($C_{24}H_{32}O_8$, m.w. = 448) has a very low absorption in the UV., indicating the saturation of the conjugated double bond. In the NMR. spectrum of **9**, the H_A and H_B protons, still coupled to H_X, are now shifted to higher field (1.59 and 2.15 δ). The most interesting feature of this spectrum is the pattern of the H_P and H_Q protons, which are now coupled both to an adjacent new proton (H_L), which is overlapped by other absorptions. From double resonance, the chemical shift of H_L is found to be 2.2 δ, which is consistent with a position α to a carbonyl.

The mass spectral behavior of wortmin (**1**) is marked by the group of peaks at *m/e* 164/165. On the basis of high resolution data and the constant appearance of these peaks in the mass spectra of wortmin derivatives the structures of the corresponding ions should be **a** and **b** respectively. The decay of the alicyclic part of this compound is not so pronounced; it appears only in the compounds **5** and **7** which are not esterified at the tertiary hydroxyl group with 2-hydroxy-4-methoxy-6-methylbenzoic acid. These two compounds do show strong fragmentation of this part of the molecule, the others show the same behavior but much less intensive. The fragmentation reaction **c** (*m/e* 280) → **d** (*m/e* 220) → **e** (*m/e* 150) for wortmin (**1**) is given in the schema 1. Shifts of the corresponding ions could be found in the spectra of the dihydro compound **8** (**d'** at *m/e* 222) and of the tetrahydro compound **9** (**d"** at *m/e* 224). **c** could be converted to **f** (*m/e* 94); again this is missing in the spectrum of **9**, because the tetrasubstituted double bond which is necessary for its forming is hydrogenated. In the spectrum of **9** an ion corresponding to **e** is not registered as expected. The intensity of peaks corresponding to **c** is sometimes very low.

An alternative structure (F) can be written for wortmin. This structure, even if unreliable on biogenetical grounds, could fit with most of the NMR. spectral data, and cannot be excluded by mass spectroscopy. In order to eliminate this uncertainty, the exchange with D of the proton near to the carbonyl group in **9** was planned.

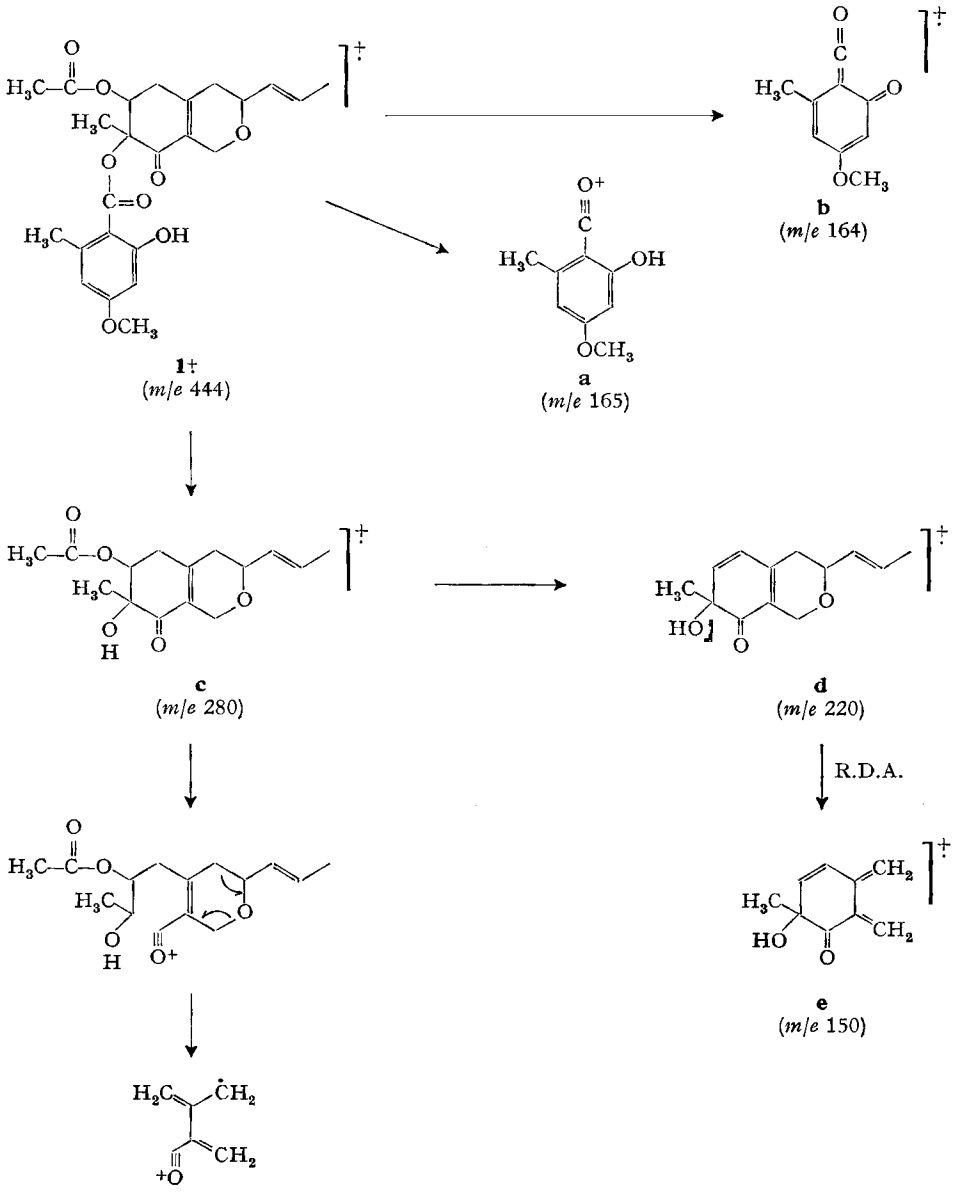


(F)

Unusual difficulties were encountered in this reaction, as treatment with D_2O in basic medium gave only degradation products, and CF_3COOD exchanged only the

aromatic protons. By eluting a solution of **9** through an alumina column pretreated with D_2O [7], the deuterated compound **10** was obtained. The exchange of H_L decoupled the protons H_P and H_Q in the NMR. spectrum, which appeared now as two sharp doublets ($J_{P,Q}$ = 12 Hz), thus establishing a link between the sequences D and E, and confirming structure **1** for wortmin.

So far lack of substance has prevented further investigation on the stereochemistry of wortmin.



Experimental Part

NMR-spectra were measured with A-60 and HA-100 *Varian* spectrometers. Spin-decoupling experiments were performed by the 'frequency sweep' method. The integral were measured with a 405/CR *Hewlett-Packard* digital voltmeter. Chemical shifts are in ppm (δ) from TMS as internal standard, J are in Hz. IR. spectra were recorded with a *Perkin-Elmer* Infracord (values in μ). UV. spectra of solutions in 95% EtOH (λ_{max} in nm) were taken with a *Beckman* DK-2 apparatus. M.p. (uncorrected) were measured with a *Kofler* apparatus. Silica gel *Merck* 0.05–0.20 mm was used for column chromatography, and *Merck* HF_{254} for TLC. Mass spectra were measured on a *CEC* instrument type 21-110B (70 eV, 8 KV direct inlet system). High resolution values were obtained by using the peak matching method. The values are given in *m/e* (rel. %).

A strain of *P. wortmanni* Klöcker, obtained from Centraalbureau vor Schimmelcultures, Baarn, was grown on oat-meal medium (Difco), in *Roux* flasks at room temp. and in daylight. After ten days, the culture from 100 flasks was extd. twice with EtOAc. The ext. (4 g) was adsorbed on silica gel and chromatographed through the same silica gel with hexane, then hexane/AcOEt 4:1. The more polar fractions contained skyrin, wortmannin, mitorubrinol (isolated by TLC. and identified by comparison with an authentic sample [5]), and flavomannin.

Wortmin (**1**) was eluted immediately after ergosterol (identified by comparison on TLC. and spectra), and obtained as small buff crystals (300 mg), m.p. 108° (hexane/ether) (Found: C, 63.81; H, 6.31; Calc. for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35%). Ms.: 444 (M^+, $C_{24}H_{28}O_8$, 2), 328 ($C_{18}H_{16}O_4$, 0.5), 302 ($C_{17}H_{18}O_5$, 3), 280 ($C_{15}H_{20}O_5$, 4), 263 ($C_{15}H_{19}O_4$, 2), 220 ($C_{13}H_{16}O_3$, 10), 210 ($C_{11}H_{14}O_4$, 10), 202 (27), 165 (93), 164 ($C_9H_8O_3$, 49), 150 (34), 138 (39), 136 (28), 134 (31), 121 (27), 107 (100), 94 (75).

Methanolysis of **1**. 200 mg of **1** in 8 ml MeOH contg. 100 mg MeONa were warmed 15 min. on a steam bath. Evapn., treatment with dil. HCl and ether extn. gave a mixture of **2**, **3** and **5**. Treatment of this mixture with CH_2N_2 and prep. TLC with hexane/EtOAc 1:1 gave *methyl 2-hydroxy-4-methoxy-6-methylbenzoate* (**3**), identified by spectra and comparison with an authentic sample [5], *methyl 2,4-dimethoxy-6-methylbenzoate* (**4**) (Ms.: 210, 179, 164; NMR. ($CDCl_3$): MeCO 2.43, 3 OMe 3.81, 3.81, 3.89; 2 arom. H 6.29), and the diol **5**. **5** is a glassy solid, UV.: 239 (ε = 8100), IR.: 2.95 (OH) and 6.0 (conj. CO) (neat), Ms.: 238 (M^+, 2), 220 (1), 195 (1), 168 (100), 150 (13), 125 (60), 108 (28), 107 (52), 95 (35), 94 (87).

Acetylation of **5** (30 mg) with 0.5 ml Py and 1 ml Ac_2O gave *diacetyl-5* (**7**). Ms.: 322 (M^+, 37), 293 (1), 280 (1), 262 (1), 237 (2), 220 ($C_{13}H_{16}O_3$, 17), 202 (26), 192 ($C_{11}H_{12}O_3$, 70), 167 ($C_9H_{11}O_3$, 90), 151 (44), 150 ($C_9H_{10}O_2$, 100), 134 (16), 133 (16), 132 (22), 125 ($C_7H_9O_2$, 24), 122 (38), 121 (44), 108 (44), 107 (C_7H_7O, 91), 94 (80).

Acid hydrolysis of **1**. 200 mg of **1** in 5 ml MeOH were added with 8 ml 5% HCl and heated on a steam bath for 8 h. Evapn., extn. with ether and prep. TLC. with hexane/AcOEt 4:1 gave *deacetylwortmin* (**6**), glassy solid, IR. (neat): 2.90, 5.95, 6.07. Ms.: 402 (M^+, 2), 280 (3), 256 (2), 238 (1), 220 (2), 182 (35), 168 (38), 165 (54), 164 (100), 150 (21), 138 (38), 125 (27), 107 (55), 94 (49). A sample of **6** gave **2** and **5** when treated with NaOMe in MeOH, as it was described for **1**.

Dihydrowortmin (**8**). Hydrogenation of 200 mg of **1** in 50 ml ether with 80 mg 10% $Pd/BaSO_4$ gave **8**, m.p. 134° (hexane/ether). UV.: 264, 303 (ε = 15800, 5300). Ms.: 446 (M^+, 2), 282 (1), 222 (10), 208 (50), 182 (23), 165 (84), 164 (100), 151 (17), 150 (14), 138 (56), 134 (54), 123 (42), 109 (39), 107 (44), 95 (60), 94 (37).

Tetrahydrowortmin (**9**). Hydrogenation of 150 mg **8** in 10 ml AcOH with 50 mg PtO_2 for 48 h., filtn., evapn., neutralization with Na_2CO_3, ether exth., and prep. TLC. of the extract (3 runs) gave unreacted **8** and **9**, as a glass. UV.: 265, 303 (ε = 2900, 1150), IR.: large band between 5.70 and 5.85. Ms.: 448 (M^+, 12), 317 (2), 278 (3), 224 (2), 208 (4), 182 (8), 165 (79), 164 (100), 138 (7), 121 (4), 109 (5).

Deuteration of **9**: 50 mg of **9** were dissolved in benzene/ether 9:1 previously satd. with D_2O, and eluted through a column of Al_2O_3 act. III, pretreated with D_2O. Evapn. gave **10**.

We thank Prof. *R. Locci* (University of Milan) for advice and assistance in microbiological work.

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13. Photochemische Reaktionen

71. Mitteilung [1]

Die Photoisomerisierung eines spirocyclischen α,β-Epoxyketons[1])

von **H. J. Wüthrich**[2a]), **A. Siewinski**[2b]), **H. Schaffner** und **Oskar Jeger**

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(6. XI. 72)

Summary. On direct UV. irradiation and on triplet sensitization with acetophenone the spirocyclic epoxyketone (*R*)-(−)-**9** undergoes racemization ($\Phi^{313/334}$ 0.014, Φ^{Sens} 0.0060) and rearrangement to the enantiomeric spiro-β-diketones (*R*)-(+)-**14** ($\Phi^{313/334}$ 0.068, Φ^{Sens} 0.0037) and (*S*)-(−)-**14** ($\Phi^{313/334}$ 0.024, Φ^{Sens} 0.0023). The quantum yield data show that triplet reaction due to intersystem crossing is unimportant on direct irradiation, and they exclude that one common diradical intermediate of type **d** (Scheme 8) for the three reaction paths is involved in both the singlet and the triplet reaction. The postulate of photolytic C_α—O epoxide cleavage to intermediates of type **d** for the rearrangement requires that the rate of rearrangement is greater than the rate of rotation around the C_α—C_β bond in a given **d**, and that the rate difference is greater in singlet-generated **d** than in the triplet analogue. Reclosure of diradicals **d** and/or photolytic C_α—C_β cleavage to diradical **e** and reclosure can account for the racemization of **9**.

The optically active spiro-β-diketone **14** was found to racemize also on direct irradiation and on triplet sensitization. Furthermore, both **14** and the isomeric β-diketone **20**, which was obtained by UV. irradiation of the homocyclic epoxyketone **19**, photochemically isomerize to the enol lactones **23** and **21**, respectively.

Einführung. Auf Grund der bisherigen Untersuchungen der Photoisomerisierung von gesättigten α,β-Epoxyketonen (**a** → **c**) an Steroiden [3] [4] sowie aliphatischen und monocyclischen Epoxyketonen [5]–[7] wurde eine zweistufige Reaktionsfolge postuliert: eine reversible homolytische C_α-O-Spaltung im photolytischen Primär-

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[1]) Ein Teil der Resultate wurde bereits in Vortragsreferaten erwähnt [2].

[2]) a) Auszugsweise der Dissertation von *H.-J. Wüthrich* entnommen (ETH Zürich, 1972). – b) Gegenwärtige Adresse: Landwirtschaftliche Hochschule, Wroclaw (Polen).