METABOLIC PRODUCTS OF MICROORGANISMS. 196* THE STRUCTURE OF ETHERICIN B, A NEW DIPHENYLETHER ANTIBIOTIC

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Ethericin B (**Ia**) was isolated together with ethericin A[1] from fermentations of *Aspergillus funiculosus* Tü 680. The structure of ethericin B was established as 4-carbethoxy-5,5′-dihydroxy-3,3′-dimethyl-diphenyl ether by chemical degradation, mass spectrometry, ^{1}H-NMR and comparison with synthetic 4-carbethoxy-5,5′-dimethoxy-3,3′-dimethyl-diphenyl ether.

Ethericin B (**Ia**) was separated from ethericin A by crystallization from methanol during the last step of the procedure described by KÖNIG *et al.*[1] Ethericin A (2,2′,3,3′-tetrahydroxy-5,5′-dimethyl-diphenyl ether) is identical with aspermutarubrol, which was identified by TANIGUCHI *et al.*[2] as a metabolite of *Aspergillus sydowi*. Ethericins A and B are inhibitory for the same test organisms under the same test conditions to a similar degree[3]; however some obvious differences were observed as follows:

	Ethericin A	Ethericin B
EDTA enhances the inhibitory effect on *Bacillus subtilis*	yes	no
Interaction with di- or polyvalent cations	yes	no
Substances absorbing at 260 and 280 nm released by cells of *B. subtilis*	no	yes

It was concluded that ethericins A and B exhibit different types of antimicrobial action at the molecular level.

Chemical Properties and Structure of Ethericin B

Ethericin B is a colourless solid substance. The UV-spectrum shows maxima at 300, 264 and 220 nm. The electron impact mass spectrum (Fig. 1) exhibits a molecular ion at *m/e* 302. According to high resolution mass spectrometry the elemental composition is $C_{17}H_{18}O_5$ (MW 302.1154). The molecular ion loses one molecule of ethanol ($M^+ - 46$) to form a fragment at *m/e* 256. This ion splits off an OH-radical to give *m/e* 239. The fragment at *m/e* 228 results from loss of CO and ethanol. The loss of ethanol is common to ethyl esters. Prolonged treatment of ethericin B with a solution of dry HCl gas in methanol (1.5 N) at 100°C results in partial transesterification to a compound of molecular weight 288. Upon trimethylsilylation with N-methyl-N-trimethylsilyl-trifluoroacetamide the molecular ion is shifted to *m/e* 446, indicating that two trimethylsilyl groups are added to the molecule. This is

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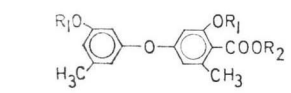

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a: R_1=H; R_2=–CH_2–CH_3
b: R_1=CH_3; R_2=–CH_2–CH_3
c: R_1=H; R_2=H

Fig. 1. Electron impact (70 eV) mass spectrum of ethericin B.

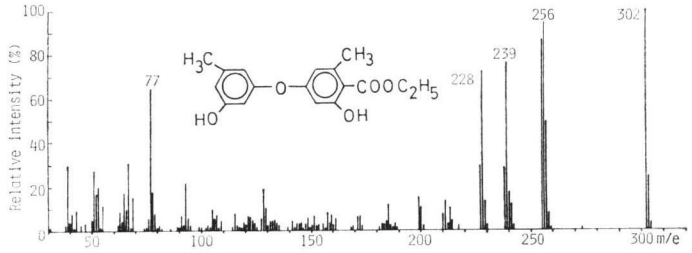
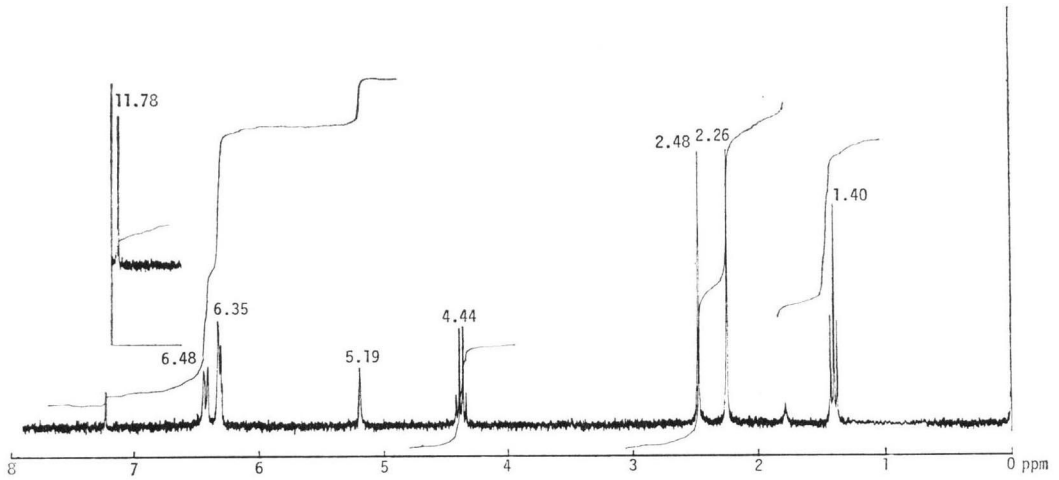


Fig. 2. ^{1}H-NMR spectrum (270 MHz) of ethericin B in $CDCl_3$ solution (TMS=internal standard).



in accordance with the presence of two hydroxy groups.

The ^{1}H-NMR spectrum in $CDCl_3$ solution (Fig. 2) of ethericin B shows a triplet at δ=1.40 (J=8.5 Hz) and a quartet at δ=4.44 (J=8.5 Hz) typical for an ethyl ester group. There are two aromatic CH_3 singlets at δ=2.26 and 2.48. The signals at δ=6.35, 6.44 and 6.48 show a total of 5 aromatic protons, which are shifted to higher field by the influence of the hydroxy substituents. The resonances at δ=5.19 and 11.78 disappear upon addition of CD_3OD to the sample, indicating that these signals may be assigned to hydroxyl protons. The low field shift of one of the hydroxyl proton signals points out that this OH group is in ortho position to the carbonyl group of the ester residue (hydrogen bonding). This assumption is supported by the strong ethanol elimination from the ester residue in the mass spectrum, which can be attributed to an ortho effect. From the high field shift of all aromatic protons it can be concluded that the other ortho position of the ethyl ester group must be occupied by a methyl group. Otherwise at least the signal of one proton in ortho position to the ester residue should be shifted to lower field.

Since there are 5 oxygen atoms in the molecular formula of ethericin B, two of which are bonded as hydroxy groups and two in the ethyl ester residue, the fifth oxygen ought to be an ether oxygen. This assumption is confirmed by cleavage of ethericin B by sodium in liquid ammonia[4]. The main cleavage product could be identified as 3,5-dihydroxy toluene by combined gas chromatography and mass spec-

trometry. These results are in accordance with the proton NMR spectrum and suggest structure **Ia** for ethericin B.

Synthesis of Bis-O-methyl Ethericin B (**Ib**)

In order to confirm the structure of **Ia**, derived by mass spectrometry and NMR, we synthesized the bis-O-methyl derivative **Ib** by a ULLMANN type reaction according to Scheme 1. The intermediate aldehyde **VI** could only be obtained in very small yield (4.9%). Also the ULLMANN reaction[5] of **VIII** and **II** and the oxidation of the resulting aldehyde **IX** with activated MnO_2 to **Ib** were accompanied by numerous side reactions. Nevertheless the product **Ib** was obtained in sufficient amounts to be compared with O-methylated natural ethericin B. The mass spectra, gas chromatographic retention times and ^{1}H-NMR spectra are completely identical and confirm the derived structure **Ia** for ethericin B.

Discussion

Diphenyl ethers are common in nature and have formerly been described from different organisms[6~8]. The structure of ethericin B (**Ia**) is closely related to a metabolite **Ic** from *Aspergillus fumigatus*, isolated and identified by YAMAMOTO and coworkers[9]. In **Ic** the carbethoxy substituent of ethericin B is replaced by a carboxy group. The same compound **Ic** was isolated from *Hypocrea citrina* by NAIR and CAREY[10].

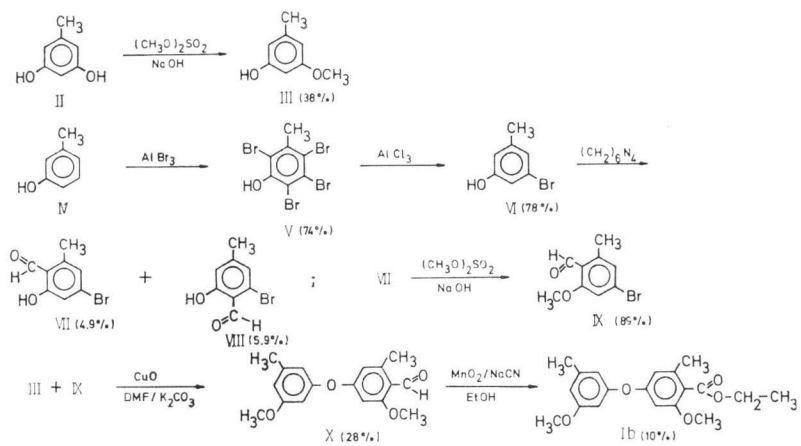
Experimental

Mass spectra were recorded on a Varian MAT CH7 instrument. For ^{1}H-NMR investigations a Bruker WH270 (270 MHz) instrument was used.

Synthesis of 2-methoxy-4-(3-methoxy-5-methyl-phenoxy)-6-methyl benzoic acid ethyl ester (**Ib**)

3-Hydroxy-5-methoxy toluene (**II**) was prepared according to HENRICH and NACHTIGALL[11]. 2,4,5,6-Tetra-bromo-3-hydroxy toluene (**V**) was obtained from 3-hydroxy toluene according to KOHN and FINK[12] and converted to 3-bromo-5-hydroxy toluene (**VI**) as described by KOHN and WEIßBERG[13]. Reaction of **VI** with hexamethylenetetramine (urotropin)[14] yielded a mixture of the aldehydes **VII** and **VIII**, which were separated by chromatography on silica gel (cyclohexane - ethyl acetate, 4: 1). Methylation of **VII** with dimethylsulfate according to PAILER and BERGTHALLER[15] gave 4-bromo-2-methoxy-6-methyl-benzaldehyde (**IX**), which was reacted with **III** together with CuO and K_2CO_3 in DMF

Scheme 1.



according to TOMITA *et al.*[16] and yielded 2-methoxy-4-(3-methoxy-5-methylphenoxy)-6-methyl-benzaldehyde (**X**). The aldehyde could be oxydized to the ethyl ester derivative **Ib** with MnO_2-NaCN in ethanol solution according to a procedure published by COREY *et al.*[17]

Methylation of ethericin B (**Ia**)

The methylation of ethericin B was performed with dimethylsulfate[15] and resulted in a product identical with **Ib** as shown by mass spectrometry and ^{1}H-NMR. MS: m/e=330 (M^+; 55%), 285 ($M-OC_2H_5$; 100%). ^{1}H-NMR ($CDCl_3$): δ 1.36 (t; CH_3), 2.24 (s; 3H, CH_3), 2.28 (s; 3H, CH_3). 3.75 (s; 6H, OCH_3), 4.36 (q; CH_2), 6.38~6.49 (m; 5H arom.)

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