Benzyl 2,6-dimethoxybenzoate (7) Colourless crystals (1 05 g), mp 64–65°, IR ν_{max}^{mull} cm^{-1} 1730, 1255, ^1H NMR δ 3 70 (6H, *s*), 5 25 (2H, *s*), 6 40 (2H, *d*, *J* = 8 Hz), 6 99–7 39 (6H, *m*), MS *m/z* (rel int) 272 $[M]^+$ (30), 165 (100), 107 (15), 91 (80) (Found C, 70 56, H, 6 08 Calc for $C_{16}H_{16}O_4$ C, 70 58, H, 5 92%)

Benzyl 2-hydroxy-5-methoxybenzoate (8) Colourless oil (105 mg), IR ν_{max}^{neat} cm^{-1} 1680, 1280; ^1H NMR δ 3 67 (3H, *s*), 5 26 (2H, *s*), 6 66–7 40 (3H, *m*), 7 30 (5H, *s*), 10 18 (1H, *s*, removed with D_2O), MS *m/z* (rel int) 258 $[M]^+$ (23), 151 (57), 107 (34), 91 (100) (Found C, 69 53, H, 5 70 $C_{15}H_{14}O_4$ requires C, 69 76, 5 46%)

Benzyl 2,5-dimethoxybenzoate (9) Colourless oil (75 mg), IR ν_{max}^{neat} cm^{-1} 1730, 1250; ^1H NMR δ 3 73 (3H, *s*), 3 76 (3H, *s*), 5 24 (2H, *s*), 6 65–6 98 (2H, *m*), 7 13–7 42 (6H, *m*), MS *m/z* (rel int) 272 $[M]^+$ (100), 165 (40), 107 (6), 91 (44) (Found C, 70 41, H, 6 11 $C_{16}H_{16}O_4$ requires C, 70 58, H, 5 92%)

Methylation of **3**, **5**, **6** *and* **8** These compounds were methylated using Me_2SO_4–K_2CO_3–Me_2CO and the products were purified by bulb-to-bulb distillation to yield **4**, **7** (from both **5** and **6**) and **9**, respectively

Syntheses of **2**–**5** *and* **7**–**9** The reaction of benzoyl chlorides (prepared from benzoic acids) with benzyl alcohols in C_6H_6 directly yielded the corresponding benzyl benzoates

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1-*N*-METHYL-(6*E*)-(2-METHYLPROPYLIDENE)-(3*Z*)-3-(PHENYLMETHYLENE)-2,5-PIPERAZINEDIONE, A METABOLITE FROM *STREPTOMYCES ALBUS*

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(*Received* 25 *May* 1983)

Key Word Index—*Streptomyces albus*, 2,5-piperazinedione derivatives

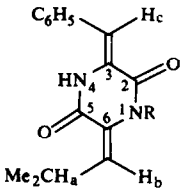
Abstract—The structure and stereochemistry of a new piperazinedione, isolated from the cells of *Streptomyces albus*, are assigned on the basis of spectroscopic data, including comparison with related 2,5-piperazinediones

In the course of our work [1] on the biosynthesis of bacterial menaquinones (vitamins K_2), the menaquinones were isolated from the non-polar lipid extract of cells of *Streptomyces albus* Further fractionation of the non-polar constituents from the bacterial cell paste afforded a hitherto unreported crystalline compound in 0 1% yield

Mass spectral analysis (found $[M]^+$, 270 1375 $C_{16}H_{18}N_2O_2$ requires 270 1368) indicated a molecular formula of $C_{16}H_{18}N_2O_2$ for the new metabolite (**1**) The UV spectrum of **1** closely resembled that of the *Streptomyces* metabolite albonoursin (**3**), [2, 3] which has a molecular formula of $C_{15}H_{16}N_2O_2$ The ^1H NMR spectrum of the metabolite (**1**) showed the presence of the 2-methylpropylidene and phenylmethylene groups The remaining signals, a three-proton singlet at δ3 27 and a one-proton multiplet at δ8 00, exchangeable with D_2O, were attributable to NMe and NH groups, respectively Thus, the gross structure of the new metabolite (**1**) differs

Table 1 ^{1}H NMR spectral data for 6-alkylidene-3-arylidene-2,5-piperazinediones (1–8)

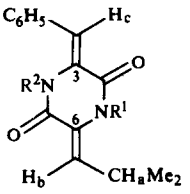
Compound	δ in $CDCl_3$			δ in CF_3CO_2H					Ref
	H_a	H_b	H_c	H_a	H_b	H_c	ArH		
1 (3*Z*, 6*E*)	3 75	5 47	6 97	3 75	6 05	7 28	7 46	*s*	
2 (3*Z*, 6*E*)				3 76	5 96	7 24	7 46	*s*	3
3 (3*Z*, 6*Z*)	2 87	6 40	7 32	2 87	6 40	7 32	7 45	*s*	3,4
4 (3*Z*, 6*Z*)	2 90	5 98	6 94						4
5 (3*Z*, 6*Z*)	2 90	6 12	7 28						4
6 (3*Z*, 6*Z*)	2 90	6 00	7 14						4
7 (3*E*, 6*Z*)				2 83	6 33	6 89	7 34	*m*	3
8 (3*E*, 6*E*)				3 75	5 87	6 83	7 42	*m*	3



R

1 Me

2 H



3 $R^1 = R^2 = H$

4 R^1 = Me, R^2 = H

5 R^1 = H, R^2 = Me

6 $R^1 = R^2$ = Me

7 $R^1 = R^2 = H$, (3*E*)− isomer

8 $R^1 = R^2 = H$, (3*E*,6*E*)− isomer

from albonoursin (**3**) in that one of the amide nitrogens is methylated

The assignment of the (3*Z*, 6*E*)-configuration to the 2,5-piperazinedione (**1**) was made by comparison of the chemical shifts of the olefinic and methine protons, and the multiplicity of the aromatic protons, with the data reported for the related 2,5-piperazinediones (**2–8**) (Table 1) [3, 4] The location of the NMe group and confirmation of the (6*E*)-configuration were obtained by a nuclear Overhauser enhancement experiment Irradiation of the singlet absorption due to the NMe group at δ 3 27 in the ^{1}H NMR spectrum of **1** gave a 16% enhancement of the doublet due to the olefinic proton (H_b) at δ 5 47

To the best of our knowledge, all naturally occurring 6-alkylidene-3-arylidene-2,5-piperazinediones possess the (3*Z*,6*Z*)-configuration, the new metabolite (**1**) thus provides the first example of a naturally occurring 2,5-piperazinedione containing an alkylidene group with the *E*-configuration

*The original isolation of the metabolite (**1**) was carried out at the Department of Biochemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pennsylvania, U S A

EXPERIMENTAL

General directions for growing *Streptomyces albus* (ATCC 3004) and harvesting the cells have been given before [1] The non-polar lipids were isolated from the cellular paste (40 g) by an Me_2CO homogenization technique, followed by partition of the extract between H_2O and Et_2O [1] The Et_2O extracts were concd and subjected to CC (silica gel, C_6H_6) to give menaquinones and ($CHCl_3$–MeOH, 10 1) 1-*N*-methyl-(6*E*)-6-(2-methylpropylidene)-(3*Z*)-3-(phenylmethylene)-2,5-piperazinedione (**1**),* 39 mg, mp 145–146° (from cyclohexane), UV λ_{max}^{MeOH} nm (ε) 230 (8770) and 317 (30 000), IR ν_{max}^{KBr} cm^{-1} 3230, 1690, and 1625 ^{1}H NMR (90 MHz, $CDCl_3$) δ 1 05 (6H, *d*, *J* = 7 Hz, 2 × Me) 3 27 (3H, *s*, NMe), 3 75 (1H, *m*, H_a) 5 47 (1H, *d*, *J* = 10 Hz, H_b), 6 97 (1H, *s*, H_c), 7 37 (5H, *s*, ArH) and 8 00 (1H, *m*, NH), (CF_3CO_2H) δ 1 16 (6H, *d*, *J* = 8 Hz), 3 48 (3H, *s*, NMe), 3 75 (1H, *m*, H_a), 6 05 (1H, *d*, *J* = 11 Hz, H_b), 7 28 (1H, *s*, H_c) and 7 46 (5H, *s*, ArH) MS (70 eV) *m/z* (rel int) 270 $[M]^+$ (100), 255 (30), 227 (10), and 82 (35) (found C, 71 0, H, 6 9, N, 10 5 $C_{16}H_{18}N_2O_2$ requires C, 71 1, H, 6 7, N, 10 4%)

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NOTE ADDED IN PROOF

We have recently been informed by Dr J P Poyser, I C I Pharmaceuticals Division, Alderley Park, Cheshire, that the same metabolite (**1**) has been isolated from a *Streptomyces* species, and the structure has been confirmed by an X-ray structure determination [A A Freer, D Gardner and J P Poyser, to be published]