A New Fungal Lactone, LL-P880β, and a New Pyrone, LL-P880γ, from a *Penicillium* sp.

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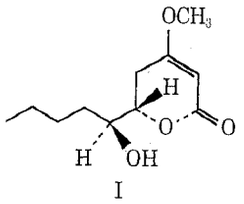
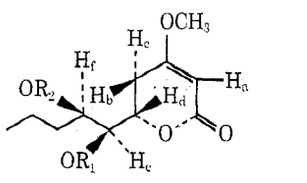
The novel metabolites LL-P880β or (6*S*,1′*S*,2′*R*)-4-methoxy-5,6-dihydro-6-(1′,2′-dihydroxypentyl)-2*H*-pyran-2-one and LL-P880γ or 4-methoxy-6-(1′,2′-dihydroxypentyl)-2*H*-pyran-2-one (as the acetate) have been isolated from an unidentified *Penicillium* sp. Use was made of the exciton chirality method to assign the stereochemistry of LL-P880β.

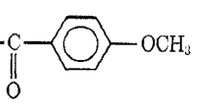
In connection with work on the antiprotozoal principles of fermentations of an unidentified *Penicillium* sp. (Lederle culture P880), we isolated the dihydropyrone I.[1] We now report the structures of two minor metabolites, II and III, from the same culture and the use of the exciton chirality method[2] to determine the stereochemistry of the vicinal glycol in II.

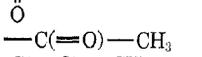


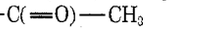
I

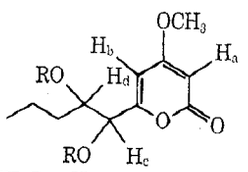


II, $R_1 = R_2 = H$

VI, $R_1 = R_2 =$ 

XI, $R_1 = H$; $R_2 =$ 

XII, $R_1 = R_2 =$ 



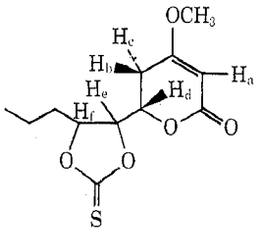
III, R = H

X, R =

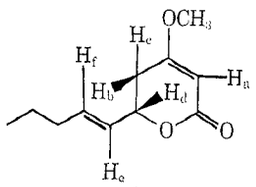
Strong ir bands at 1705 and 1640 cm^{-1}, a uv maximum at 234 nm (ε 13,800), and an nmr methoxyl signal at δ 3.80 in the various spectra of LL-P880β indicate that the unsaturated δ-lactone of I exists intact in this metabolite. A major fragmentation in the mass spectrum of the material results from the loss of a propyl group to give the peak *m/e* 187. Another large peak at *m/e* 127 is consistent with the loss of $C_5H_{11}O_2$ thus establishing II as the gross structure of this compound.

Refluxing of II with thiocarbonyldiimidazole in toluene[3] gives the 1,2-thionocarbonate IV. Compound IV undergoes cis elimination in refluxing trimethyl phosphite to give the olefin V. The coupling constant J_{ef} = 16 Hz in the nmr spectrum of V establishes the trans nature of this olefin.

A CD curve of II shows $\Delta\epsilon_{246} = -12.5$ indicating



IV



V

that the asymmetric center of the lactone ring has the same *S* configuration as in I which exhibited $\Delta\epsilon_{243}$ −7.9. The stereochemistry of the asymmetric centers at $C_{1'}$ and $C_{2'}$ is represented by one of the four possibilities: *SSS*, *SRR*, *SRS*, and *SSR*. The erythro isomers *SSS* and *SRR* may be discounted on the basis of the trans olefin V obtained as mentioned by the Corey procedure.

A dianisoate, VI, of II was prepared and the CD curve on this material shows two strong Cotton effects of opposite sign at 267.5 nm ($\Delta\epsilon$ −19.0) and 250 nm ($\Delta\epsilon \simeq +18.6$ when corrected for the nullifying effect of the enelactone Cotton effect at 243 nm). The location and magnitude of these effects clearly indicate that we are dealing with the Davydov-split Cotton effects of the *p*-methoxybenzoate chromophore.[4] Thus, on the basis of the exciton chirality method, a left-handed helix is indicated for the $C_{1'}$–$C_{2'}$ glycol arrangement.

An average value for the coupling constants of cis glycol protons in the gauche conformation is approximately 4.0 Hz while the average value for these protons in the trans conformation is about 8.0 Hz.[5,6]

It is quite conceivable that these values could be decreased due to the protons in question being squeezed closer together by bulky substituents. It is unreasonable to suggest that either approximate value would be markedly increased due to presence of bulky groups. Consequently, the observed value J_{ef} = 6.0 Hz for the glycol protons of VI means that they are trans to one another. The Newman projections shown below depict the trans conformations of the possible isomers *SRS* and *SSR* of II together with the kind of Cotton effect expected from each.

(1) G. A. Ellestad, W. J. McGahren, and M. P. Kunstmann, *J. Org. Chem.*, **37**, 2045 (1972).

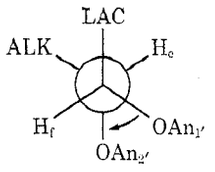
(2) N. Harada and K. Nakanishi, *Accounts Chem. Res.*, **5**, 257 (1972).

(3) E. J. Corey, F. A. F. A. Carey, and R. A. E. Winter, *J. Amer. Chem. Soc.*, **87**, 934 (1965).

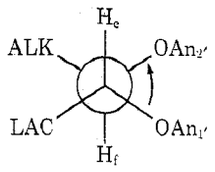
(4) N. Harada and K. Nakanishi, *Accounts Chem. Res.*, **5**, 258 (1972).

(5) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, New York, N. Y., 1969, p 292.

(6) H. El. Hkadem, D. Horton, and J. D. Wander, *J. Org. Chem.*, **37**, 1630 (1972).



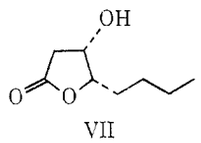
SRS positive
Davydov-split
Cotton effect



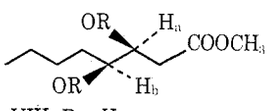
SSR negative
Davydov-split
Cotton effect

Conceivably, two of the groups, *i.e.*, the lactone and alkyl groups, have greater steric requirements so that the trans protons are skewed to a dihedral angle smaller than 180° which in turn reduces the value of the trans coupling constant to the observed value of 6 Hz. It follows then that *SSR* is the configuration of II.

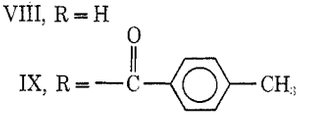
The exciton chirality method fails, of course, when the predominant rotameric population is such that the two anisoate groups are separated by about 180°. We observed this situation in regard to a degradation product of I. Ozonolysis of I yields mostly the lactone VII and also a small quantity of the cis glycol ester VIII. Because of its origin from I, the stereochemistry of VIII must be *SS*.



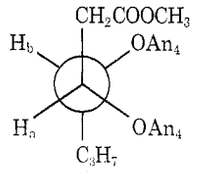
VII



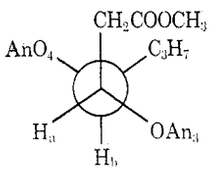
VIII, R = H



The dianisoate IX was prepared and in the nmr spectrum of this derivative the C_2 methylene protons appear as a doublet at δ 2.76 while H_a and H_b are represented by virtually identical multiplets at δ 5.70 and 5.40. Irradiation of the δ 2.76 doublet collapses the δ 5.70 multiplet to a doublet revealing the vicinal coupling constant J_{ab} = 3.5 Hz, indicating a gauche conformation to be predominant. Two gauche conformers may be drawn.



negative Davydov-split
Cotton effect



small normal anisoate
Cotton effect

In both these conformers, the massing of all the bulky groups together would tend to squeeze the H_a and H_b protons closer together thus accounting for the smaller than average gauche coupling constant of 3.5 Hz. The CD curve of IX shows a very small positive Cotton effect at 280 nm ($\Delta\epsilon$ +0.90) and a larger effect at 252 nm ($\Delta\epsilon$ −6.2); consequently, the preferred conformer is that shown on the right-hand side above since, when the dihedral angle between the anisoate groups is 180°, Davydov-splitting effects are absent.[7]

It is unexpected to find the gauche conformer predominate, but recently Birdsall, *et al.*,[8] showed that the gauche conformers of the glycol fragment of dipalmitoyllecithin represented about 37% of the total rotamer population.

Partition chromatography of the filtrate concentrate from which II was recovered yielded a crystalline solid, mp 114–115°, which by tlc was a single uv-absorbing spot. However, neither carbon–hydrogen analysis nor mass spectroscopy data gave a satisfactory molecular formula for the material. Under mild conditions, the material was acetylated to reveal two compounds on examination of the reaction mixture by tlc. The less polar material had a uv maximum at 280 nm (ε 6240) consistent with that of an α-pyrone[9] and the nmr confirmed this compound to be X. The more polar material proved to be the monoacetate XI. In the nmr spectrum of XI, irradiation at δ 5.09–5.11 (H_f and H_a) affects protons H_e, H_b, and the farthest downfield methylene protons of the propyl moiety.

Allylic coupling of H_a to H_c is superimposed on the vicinal coupling of H_f to H_e and the aforementioned methylene group. Irradiation of δ 3.60 (H_e) causes changes in the signals of H_d and H_f indicating a vicinal coupling to both. Irradiation at δ 4.43 (H_d) shows changes in H_e, H_b, and H_c again revealing the vicinal couplings. From these studies the confirmed coupling constants are as follows: J_{bc} = 17 (geminate), J_{cd} = 12 (trans), J_{bc} = 4 (cis), J_{ac} ~2 (allylic), J_{ed} = 4 (vicinal), J_{ef} = 4 (vicinal), and $J_{f,CH_2} \simeq 6.5$. In the spectrum of II, the H_f signal is hidden under the methoxy signal at δ 3.80 whereas, in that of the monoacetate, it is shifted to δ 5.20 which is, of course, diagnostic of the acetylation of a secondary alcohol. Acetylation of the cocrystallized mixture of II and III under normal conditions (pyridine and acetic anhydride) yields the diacetate X and XII.

Experimental Section

Tlc was carried out using Brinkmann silica gel thin layers. Solvents were dried overnight over 3A or 4A molecular sieves. Solutions were dried for 15–30 min over anhydrous $MgSO_4$. Uv curves were made on a Cary 11 instrument, ir spectra were recorded on a Perkin-Elmer Infracord, and nmr spectra were made using a Varian HA-100 spectrophotometer. CD spectra were made on a Cary 60 spectropolarimeter with CD attachment. All melting points are uncorrected and were obtained in capillary tubes. Mass spectra were run on an AE I MS9 high-resolution, direct-inlet mass spectrometer.

Isolation of II.—A 300-l. fermentation tank of Lederle culture P880 was processed by extraction of the whole mash with 0.5 vol. of ethyl acetate. Concentration of the extract to about 3 l. of dark red solution followed, which, on being allowed to remain in the cold room for 2 days, yielded 24 g of pure I. By replacing the ethyl acetate of the filtrate with ether, a further 27 g of I were recovered. The filtrate from this recovery was concentrated and allowed to sit for 24 hr to yield 3 g of II which was recrystallized from ethyl acetate–hexane to obtain 2.3 g of pure white crystals of II: mp 135.5–136°; $[\alpha]^{25}D$ −59.8 ± 1.0° (c 0.96, MeOH); λ_{max}^{MeOH} 234 nm (ε 13,800); ir (KBr) 1710 and 1624 cm^{-1}; nmr ($CDCl_3$) δ 0.93 ($-CH_2CH_3$, t), 1.50 ($(CH_2)_2CH_3$, m), 2.36 (H_c, dd, J_{cb} = 17.5, J_{cd} = 12.5 Hz), 2.87 (H_b, qq, J_{bd} = 4 Hz), 3.47 (H_e, dd, J_{ef} = 3.8, J_{ed} = 4.2 Hz), 3.78 (H_d, m, hidden by OCH_3 signal), 3.80 (OCH_3, s), 4.51 (H_d, tt), 5.16 (H_a, d, J_{ac} ~ 2 Hz); CD (MeOH) $\Delta\epsilon_{246}$ −12.5, $\Delta\epsilon_{220}$ +6.6, shoulder at $\Delta\epsilon_{210}$ +4.2 (concn 0.236 mg/ml, 1 mm cell, and 0.1° full scale deflection); mass spectrum *m/e* 230.

Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.38; H, 7.88. Found: C, 57.02; H, 7.76.

(7) N. Harada, H. Sato, and K. Nakanishi, *Chem. Commun.*, 1691 (1970).

(8) N. J. Birdsall, J. Feeney, A. G. Lee, Y. K. Levine, and J. C. Metcaffe, *J. Chem. Soc.*, 1441 (1972).

(9) A. I. Scott, "Ultraviolet Spectra of Natural Products," Pergamon Press, New York, N. Y., 1964, p 141.

Attempted Isolation of III.—The filtrate from which II was recovered was concentrated to a gum and subjected to silica gel chromatography using a gradient of ethyl acetate in hexane to obtain a further 12 g of I and 4.5 g of a mixture. Partition chromatography of this mixture over acid-washed diatomaceous earth using hexane–ethyl acetate–MeOH–H_2O as 70:30:15:6 yielded 2.0 g of pure II and 420 mg (appeared as a single spot on tlc) which upon recrystallization from ethyl acetate–hexane yielded 320 mg of beautiful white crystals; mp 114–115°; $[\alpha]^{25}D$ −27.8 ± 1.2° (*c* 0.82, MeOH); λ_{max}^{MeOH} 232, 280, and 320 nm (ratio of OD's 15:3:1).

Anal. Found: C, 57.45; H, 7.51.

About 70 mg of this material was dissolved in 4 ml of Ac_2O and 100 mg of anhydrous NaOAc added. The suspension was allowed to sit overnight. Work-up of the reaction by silica gel chromatography yielded 23 mg of white crystals which spectral data showed to be X: mp 125–126°; $[\alpha]^{25}D$ 0 ± 2.6° (*c* 0.37, MeOH); λ_{max}^{MeOH} 220 nm (ε 2000) and 280 (6240); nmr ($CDCl_3$) δ 0.90 (–$(CH_2)_2CH_3$, t), 1.45 (–$(CH_2)_2CH_3$, m), 2.05 (–C(=O)–CH_3, s), 2.17 (–C(=O)–CH_3, s), 3.80 (–OCH_3, s), 5.40 (H_b, H_c, and H_d, m), 5.95 (H_a, d, $J_{ab} \simeq 1.5$ Hz); mass spectrum *m/e* 312.

Approximately 10 mg of another crystalline material were also obtained, mp 116–117°. The nmr of this material, XI, which is the $C_{2'}$ monoacetate of II, is discussed in the body of the text, mass spectrum *m/e* 272.

Acetylation of the cocrystallized material, mp 114–115°, using pyridine and Ac_2O yielded the diacetate X and also the diacetate of II or XII which melts at 87–88°: nmr ($CDCl_3$) δ 0.90 (–$(CH_2)_2CH_3$, t), 1.50 (–$(CH_2)_2CH_3$, m), 2.09 (–C(=O)–CH_3, s), 2.16 (–C(=O)–CH_3, s), 5.40 (H_c, dd, J_{cb} = 17, J_{cd} = 10.4, Hz), 2.56 (H_b, qq, J_{bd} = 5.2 Hz), 3.75 (–OCH_3, s), 4.53 (H_d, qq), 5.14 (H_a, d, $J_{ac} \sim 1.2$ Hz), 5.20 (H_e and H_f, m, J_{ef} = 4.3 Hz).

Preparation of IV.—About 220 mg (1 mmol) of II was refluxed for 1 hr with 370 mg of thiocarbonyldiimidazole in 25 ml of dry toluene. Work-up of reaction using silica gel chromatography yielded 180 mg of an oil which crystallized from ether: mp 86–87°; nmr ($CDCl_3$) δ 0.98 (–$(CH_2)_2CH_3$, t), 1.60 (–$(CH_2)_2CH_3$, m), 2.36 (H_c, dd, J_{cb} = 17, J_{cd} = 13 Hz), 2.92 (H_b, qq, J_{bc} = 5.2 Hz), 3.77 (–OCH_3, s), 4.50 (H_d and H_e, m), 5.06 (H_f, q, $J_{ef} \sim 7.5$ Hz), 5.16 (H_a, d, $J_{ac} \sim 2$ Hz); mass spectrum *m/e* 272.

Note the value J_{ef} = 7.5 Hz makes it difficult to say whether these protons are cis or trans, a difficulty also encountered with cyclic carbonates. Recently Nakanishi, *et al.*, have differentiated between threo and erythro isomers of these types of compounds using the NOE effect.[10]

Preparation of V.—About 150 mg of VI were refluxed for 90 hr in 25 ml of trimethyl phosphite in a N_2 atmosphere. The trimethyl phosphite was evaporated off under reduced pressure, and the oily residue was purified over silica gel to yield 140 mg of a smelly oil. This was purified further by partition chromatography over diatomaceous earth using the system heptane–MeOH to obtain 60 mg of V: nmr ($CDCl_3$) δ 0.90 (–$(CH_2)_2CH_3$, t), 1.50 (–$CH_2CH_2CH_3$, m), 2.10 (–$CH_2CH_2CH_3$, m), 2.40 (H_b and H_c, m), 3.75 (–OCH_3, s), 5.07 (H_a virtual s), 5.50 (H_e, dd, J_{ef} = 16, $J_{ed} \sim 5$ Hz), 5.75 (H_f, m); mass spectrum *m/e* 196.

Preparation of VI.—Approximately 1 g of II in 5 ml of dry pyridine with 1 ml of freshly distilled *p*-methoxybenzoyl chloride added was allowed to sit for 3 days. Ice–water was added and the product extracted with ether; the pyridine and anisic acid impurities were removed by extraction with 4 *N* HCl and 7% K_2CO_3, respectively. Purification by both adsorption and partition chromatography followed to get 1.2 g of a gummy solid which failed to crystallize. By evaporation of solvent from a methanolic solution of the material, a white solid, mp 66–72°, could be obtained: λ_{max}^{MeOH} 225 nm (ε 38,300); nmr (CCl_4) δ 0.92 (–CH_2CH_3, t), 1.40 (–$CH_2CH_2CH_3$, m), 1.72 (–$CH_2CH_2CH_3$, m), 2.40 (H_c, dd, J_{cb} = 17, J_{cd} = 10 Hz), 2.5 8(H_b, dd, J_{bd} = 5.0 Hz), 3.63 (–OCH_3, s), 3.78 (2 aromatic –OCH_3, s), 4.63 (H_d, m), 4.98 (H_a, s), 5.50 (H_e and H_f, m, J_{de} = 4, J_{ef} = 6 Hz), 6.77 (4 aromatic protons ortho to –OCH_3, dd, $J \sim 9$ and 1.5 Hz), 7.88 (4 aromatic protons ortho to carbonyl, m); CD (MeOH) $\Delta\epsilon_{287.5}$ −19.0, $\Delta\epsilon_{250}$ +6.7, $\Delta\epsilon_{219}$ −4.9 (between maxima at 235 and 219 nm there was no crossover, just a minimum at 225 nm), $\Delta\epsilon_{211}$ +9.8 (concn 0.240 mg/ml, 1-mm cell, and full scale deflection setting 0.1°); mass spectrum *m/e* 498.

Anal. Calcd for $C_{27}H_{30}O_9$: C, 65.05; H, 6.08. Found: C, 64.61; H, 6.00.

Ozonolysis of I.—About 6 g of I were dissolved in 175 ml of MeOH, cooled to −78°, and subjected to a stream of O_2–O_3 from a Welsbach generator for 1.5 hr. The reaction solution was worked up using 5 ml of dimethyl sulfide and the products were resolved by silica gel chromatography. These included 320 mg of VIII: mp 54°; $[\alpha]^{25}D$ −32.1 ± 1.0° (*c* 0.98, MeOH); nmr ($CDCl_3$) δ 0.93 (–$(CH_2)_3CH_3$, m), 1.44 (–$(CH_2)_3CH_3$, m), 2.59 (–CH_2COOCH_3, d), 2.74 (2 exchangeable OH's), 3.46 (–CH(OH)–CH_2COOCH_3).

Anal. Calcd for $C_9H_{18}O_4$: C, 56.82; H, 9.52. Found: C, 57.01; H, 9.34.

Surprisingly, after VIII had been eluted off the column, a band was eluted off to yield about 4.0 g of crude lactone VII. A sample of the lactone was purified first by partition chromatography and then thick layer chromatography to get the analytical sample, $[\alpha]^{25}D$ −98.2 ± 1.3 (*c* 0.77, MeOH).

The ir and nmr of this material were identical with the corresponding curves on the same compound obtained as described in ref 1. However, the $[\alpha]^{25}D$ values are different as that of the latter material was −71.1° (*c* 0.73, MeOH).

Preparation of IX.—About 85 mg of VIII were dissolved in 0.4 ml of dry pyridine and 0.3 ml of anisoyl chloride and left for 3 days. Work-up yielded 55 mg of an oil which by nmr data was clearly IX: $[\alpha]^{25}D$ −98.2 ± 1.3° (*c* 0.77, MeOH); nmr ($CDCl_3$) δ 0.87 (–$(CH_2)_3CH_3$, t), 1.35 (–$CH_2(CH_2)_2CH_3$, m), 1.72 (–$CH_2(CH_2)_2CH_3$, m), 2.76 (–CH_2COOCH_3, d), 3.63 (–$COOCH_3$, s), 3.87 (2 aromatic –OCH_3, s), 5.40 (H_a, m), 5.70 (H_b, m), 6.93 (4 aromatic H's, dd), 8.02 (4 aromatic H's, m), J_{ab} = 3.5 Hz (determined by spin decoupling); CD (MeOH) $\Delta\epsilon_{290}$ 0, $\Delta\epsilon_{280}$ +0.9, $\Delta\epsilon_{272}$ 0, $\Delta\epsilon_{252}$ −6.18, $\Delta\epsilon_{231}$ 0 (concn 0.15 mg/ml, cell width 1 mm, 0.040 full scale deflection setting); mass spectrum (molecular ion) *m/e* 458.

Acknowledgments.—Our thanks are due to Mr. L. Brancone and associates for microanalytical data, to Mr. W. Fulmor and associates for spectral data and optical rotations, to Mr. A. J. Shay for the 300-l. fermentation tank, and to Mr. M. Dann for the extraction and concentration of this tank.

Registry No.—II, 41164-59-4; III, 41206-21-7; IV, 41164-60-7; V, 41164-61-8; VI, 41164-62-9; VII, 34565-37-2; VIII, 41164-64-1; IX, 41164-65-2; X, 41164-66-3; XI, 41164-67-4; XII, 41164-68-5.

(10) K. Nakanishi, D. A. Schooley, M. Koreeda, and I. Miura, *J. Amer. Chem. Soc.*, **94**, 2867 (1972).