Synthesis of pencolide and corroboration of its revised stereochemistry

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A synthesis of the fungal metabolite pencolide from citraconic anhydride and threonine is described. Evidence is presented which corroborates the *Z* configuration recently assigned to the metabolite.

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On décrit la synthèse du métabolite pencolide à partir de l'anhydride citraconique et de thréonine. On présente des évidences qui confirment la configuration *Z* récemment attribuée au métabolite.

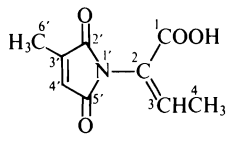
Isolation of the metabolite pencolide, **1**, from cultures of *Penicillium multicolor* Grigorieva-Manilova and Poradielova (N.R.R.L. 4036) was reported by Birkinshaw *et al.* in 1963 (1). The *E* configuration was subsequently assigned to the double bond on the basis of pmr arguments (2), however Olsen and collaborators have revised the geometry to *Z* in a recent publication (3). It was suggested (1) that the biosynthetic precursors of pencolide might be citraconic acid and threonine or a related amino acid.

We describe herein a facile synthesis of the metabolite, based on this biosynthetic concept, as well as additional evidence which corroborates the revised geometry (**2**, R = OH) of pencolide (3).

The synthesis was effected by heating citraconic anhydride with L-threonine (or racemic *allo*-threonine) at 150 °C without a solvent. Chromatography of the product furnished, in yields greater than 40%, a crystalline compound identified as pencolide on the basis of its physical and chemical characteristics.[2]

Reexamination of the reaction of the metabolite with diazomethane, first described in the original study (1), has provided independent support for the amended (*Z*) stereochemistry. Treatment of pencolide with this reagent "until effervescence ceased" was reported to result in "addition of diazomethane, presumably to a double bond, in addition to methylation" (esterification) (1). On repeating the reaction it was found that the simple methyl ester **2** (R = OMe) or the pyrazoline ester **3** (*cf.* ref. 4) could be obtained by using the appropriate amounts of diazomethane (excess reagent resulted in further attack at the Δ^2 double bond.

While the spectra of **2** (R = OMe) were unremarkable, a recrystallized, analytically pure sample of pyrazoline **3**, $C_{11}H_{13}N_3O_4$ (apparently homogeneous on tlc) showed broadening and duplication of signals in the 220 MHz pmr spectrum; based on the methyl ester signals, the presence of two isomers in the approximate ratio 14:10 was indicated. That the doubling of signals



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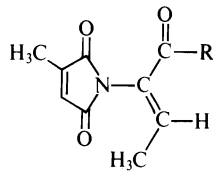

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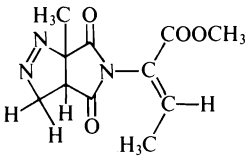

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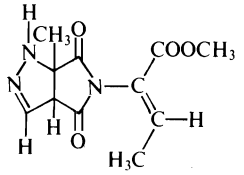

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was not due to loss of stereochemical integrity of the Δ^2 double bond, but rather to conformational effects, became clear from variable temperature studies. Thus, when the spectrum was run at −50 °C, peaks were dramatically sharpened and complete first-order analysis of the two interlacing spectra (ratio now *ca.* 18:10) became possible (5). Furthermore, the spectrum of the isomerized pyrazoline **4** (*vide infra*) at ambient

[1]Revision received May 10, 1976.

[2]Direct comparison of synthetic material with an authentic sample of pencolide kindly provided by Professor Sir Derek Barton confirmed their identity.

temperature similarly displays duplication of signals associated with conformational isomerism, and these signals coalesced in a spectrum recorded at 50 °C.

In the low temperature spectrum of **3**, the signals corresponding to the C-4 methyl and the methyl ester hydrogens of the preferred conformer occur respectively at δ 1.59 and 3.80 ppm, while for the minor conformer, these are observed at δ 1.82 and 3.70 ppm. Significantly, the chemical shifts of the C-3 olefinic hydrogen do not differ appreciably in the major and minor conformers (overlapping quartets at δ 7.32 and 7.37 ppm respectively).

Inspection of space-filling (Courtauld's) models of pencolide derivatives indicates considerable non-bonded interaction between double bond substituents and the imide oxygen atoms on rotation about the N-1′, C-2 bond, *i.e.* a substantial energy barrier can be predicted for interconversion of the rotamers. Somewhat similar phenomena are well documented for *N′,N′*-diacyl-*N*-amino imides (6). This suggests that the duplication of signals in the spectrum of **3** is a manifestation of such restricted rotation, and it is apparent that the 0.23 ppm separating the signals for the C-4 hydrogens in the two conformers results from shielding of this methyl group, in the preferred conformation, by the pyrazoline system. A chemical shift difference of 0.10 ppm for the methyl ester signals of the major and minor rotamers, with the *latter* appearing upfield, is in consonance with this interpretation. Accordingly, the relative constancy of the C-3 olefinic proton quartet demonstrates that the latter hydrogen is outside the shielding influence of the pyrazoline, and the *Z*-configuration, **2** (R = OH) is indicated for pencolide, in agreement with Olsen's assignment (3).

On heating **3** in chloroform solution under reflux, it underwent gradual rearrangement to **4** (the reaction appeared to be accelerated by the presence of traces of acid). In the 220 MHz pmr spectrum of **4**, restricted rotation was again manifested as doubling of some signals (ratio *ca.* 19:10). The duplicate signals (specifically those due to the methyl ester and the C-4 methyl protons) coalesced above ambient temperature as previously noted. Although the long-range shielding effect associated with the pyrazoline system of **4** is smaller in the region of interest than that of **3**, the C-4 methyl doublet for the major rotamer still appears slightly upfield from that of the minor conformer. The chemical shift of the C-3 olefinic proton (coincident quartets for the two conformers) is again essentially unaffected by the pyrazoline ring, behavior consistent with the assigned *Z*-stereochemistry.

Experimental

Melting points were determined on a hot-stage apparatus and are uncorrected. Infrared spectra were recorded on a Beckmann IR-10 spectrophotometer. Ultraviolet spectra were obtained using a Perkin-Elmer 402 spectrophotometer. Nuclear magnetic resonance spectra at 60 MHz were recorded for solutions in deuteriochloroform with a Varian T-60 instrument employing tetramethylsilane as internal standard; the 220 MHz nmr spectra were measured on a Varian HR-220 instrument at the Canadian 220 MHz NMR Center, Ontario Research Foundation. Mass spectra were obtained with an Hitachi–Perkin-Elmer RMU-6D mass spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Synthesis of Pencolide

A mixture of L-threonine (300 mg, 2.52 mmol) and citraconic anhydride (0.35 ml, 436 mg, 3.89 mmol) was heated with stirring at 150 °C for 3 h. Preparative layer chromatography of the resulting brown oil on silica gel plates (benzene–acetone, 4:1) afforded crude pencolide, which crystallized from ether–*n*-hexane to give 208 mg colorless crystals (42% based on L-threonine), mp 133–138 °C; ir (KBr) *inter alia*, 2600–2200, 1780(w), 1715, 1690, 1650, 1405, 1380, 1330, and 1300 cm^{-1}; ir ($CHCl_3$) 3600–2200, 1780(w), 1720, and 1660 cm^{-1}; uv λ_{max} (H_2O) 215 nm (ϵ19 200); pmr ($CDCl_3$, 60 MHz) δ 1.83 (3H, d, $J \sim 7$ Hz), 2.16 (3H, d, $J \sim 2$ Hz), 6.52 (1H, q, $J \sim 2$ Hz), 7.48 (1H, q, $J \sim 7$ Hz), 11.37 (1H, br s); mass spectrum, *inter alia*, *m/e* 195 ($M^{\ddagger}$), 177 (base peak) $(M - H_2O)^{\ddagger}$. *Anal.* calcd. for $C_9H_9NO_4$: C 55.38, H 4.65, N 7.18; found: C 55.46, H 4.71, N 7.26.

In the same manner *allo*-threonine (racemic) (280 mg, 2.35 mmol) and citraconic anhydride (0.3 ml, 374 mg, 3.34 mmol) gave 190 mg (41%) of crystalline pencolide indistinguishable from the above material.

Direct comparison of synthetic material with an authentic sample of natural pencolide confirmed their identity (see footnote 2).

Reaction of Pencolide with Diazomethane (cf. ref. 1).

Treatment of a suspension of pencolide in ether with an ethereal diazomethane solution of known concentration gave either the simple methyl ester **2** (R = OMe) or the pyrazoline ester **3** depending on the molar ratios employed. The ester **2** (R = OMe) (which was also prepared by treatment of the acid chloride with methanol) was obtained as a colourless oil from chromatography of the crude product on preparative layer plates of silica gel; ir ($CHCl_3$) 1780(w), 1720, and 1665 cm^{-1}; nmr ($CDCl_3$, 60 MHz) δ 1.79 (3H, d, $J \sim 7$ Hz), 2.16 (3H, d, $J \sim 2$ Hz), 3.79 (3H, s), 6.52 (1H, q, $J \sim 2$ Hz), 7.34 (1H, q, $J \sim 7$ Hz); mass spectrum, *inter alia*, *m/e* 209 ($M^{\ddagger}$), 177 $(M - CH_4O)^{\ddagger}$, 96 (base peak) $(C_5H_4O_2)^{\ddagger}$, and 68

$(C_4H_4O)^+$. *Mol Wt.* calcd. for $C_{10}H_{11}NO_4$:209.0688; found (M^+): 209.0688.

In the cycloaddition reaction, the pyrazoline ester **3** could be obtained without chromatography in 42% yield by crystallization of the crude product from ether–*n*-hexane: colorless crystals, mp 117–120 °C, ir (KBr) *inter alia*, 1795(w), 1735(sh), 1725, and 1665 cm^{-1}; uv λ_{max}(EtOH) <210 nm; nmr ($CDCl_3$, 220 MHz) δ 1.57 (0.60 methyl, d, $J \sim 7$ Hz), 1.78 and 1.80 [1.42 methyl, d (at δ 1.79) $J \sim 7$ Hz, superimposed on singlet(s)], 2.99–3.12 (1H, m), 3.69 (0.42 methyl, s), 3.79 (0.58 methyl, s), 4.84–5.21 (2H, 8 peak m), 7.22–7.36 (1H, 5 peak m); pmr ($CDCl_3$, 220 MHz, −50 °C) (peaks sharpened and resolved: overlapping spectra corresponding to two rotamers, ratio *ca.* 18:10); *major rotamer*: δ 1.59 (d, $J \sim 7$ Hz, C-4 methyl), 1.81? (s, angular methyl), 3.19 (dd, $J \sim 9.5$ and 2.5 Hz, X part of AMX), 3.80 (s, ester methyl), 4.95 (dd, $J \sim 19$ and 9.5 Hz, M part of AMX), 5.24 (dd, $J \sim 19$ and 2.5 Hz, A part of AMX), 7.32 (q, $J \sim 7$ Hz, C-3 hydrogen); *minor rotamer*: δ 1.82 (d, $J \sim 7$ Hz, C-4 methyl), 1.84? (s, angular methyl), 3.10 (dd, $J \sim 9.5$ and 3.5 Hz, X part of AMX), 3.70 (s, ester methyl), 5.04 (dd, $J \sim 19$ and 9.5 Hz, M part of AMX), 5.21 (dd, $J \sim 19$ and 3.5 Hz, A part of AMX), 7.37 (q, $J \sim 7$ Hz, C-3 hydrogen); mass spectrum, *inter alia*, *m/e* 251 (M^+), 223 probably $(M - N_2)^+$, 191 (base peak) $(M - N_2 - CH_4O)^+$. *Anal.* calcd. (sample mp 110–113 °C) for $C_{11}H_{13}N_3O_4$: C 52.58, H 5.22, N 16.73; found: C 52.66, H 5.27, N 16.71.

Pyrazoline **4**

A solution of pyrazoline **3** (42 mg, 0.167 mmol) in chloroform (10 ml) containing *p*-toluenesulfonic acid (0.5 mg) was heated under reflux for 12 h. The resulting solution was washed with brine, dried over magnesium sulfate, and evaporated to dryness, furnishing **4** as a colorless oil in quantitative yield: ir ($CHCl_3$) 3380, 1795(w), 1730 and 1665 cm^{-1}; uv λ_{max}(EtOH) <210 nm; nmr ($CDCl_3$, 220 MHz) δ 1.68 and 1.72 (1.65 methyl, s at δ 1.68, superimposed on doublet (δ 1.70), $J \sim 7$ Hz), 1.77 (0.35 methyl, d, $J \sim 7$ Hz), 3.74 (0.35 methyl, s), 3.79 (0.65 methyl, s), 4.04 (1H, br s with shoulder at δ 4.02), 6.30 and 6.40 (together 1H, br singlets, exchangeable with D_2O), 6.79 (1H, d, $J \sim 2$ Hz), 7.32 (1H, q, $J \sim 7$ Hz); in a spectrum run at 50 °C, the duplicate signals corresponding to the C-4 methyl group and the methyl ester protons coalesced to a doublet ($J \sim 7$ Hz) and a singlet respectively; mass spectrum, *inter alia*, *m/e* 251 (base peak) (M^+), 223 (*ca.* 6%) probably $(M - N_2)^+$, 191 $(M - N_2 - CH_4O)^+$. *Mol. Wt.* calcd. for $C_{11}H_{13}N_3O_4$: 251; found: *m/e* 251.

Acknowledgments

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1. J. H. Birkinshaw, M. G. Kalyanpur, and C. E. Stickings. Biochem. J. **86**, 237 (1963).
2. A. G. Brown and T. C. Smale. Chem. Commun. 1489 (1969).
3. A. Srinivasan, K. D. Richards, and R. K. Olsen. Tetrahedron Lett. 891 (1976).
4. W. I. Awad, S. M. Abdel, R. Omran, and M. Sobhy. J. Org. Chem. **26**, 4126 (1961).
5. L. M. Jackman and S. Sternhell. Applications of nuclear magnetic resonance spectroscopy in organic chemistry. Pergamon Press, Oxford. 1969.
6. B. H. Korsch and N. V. Riggs. Tetrahedron Lett, 5897 (1966); S. M. Verma and C. K. Rao. Tetrahedron, **28**, 5029 (1972); S. M. Verma, K. O. P. Sinha, and C. K. Rao. Can. J. Chem. **52**, 2399 (1974).