Microbiological Reduction of 2-Methyl-1,2-di-3-pyridyl-1-propanone (Metyrapone)

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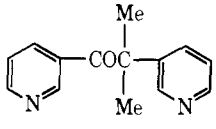
2-Methyl-1,2-di-3-pyridyl-1-propanone (metyrapone) is reduced to the (−) isomer of the corresponding alcohol, metyrapol, in 90% yield and with ~99% stereospecificity by the organism *Botryodiplodia theobromae* Pat. The (+) isomer of metyrapol has been obtained by chemical resolution of the racemate. In preliminary experiments on the inhibition of hydroxylation of desoxycorticosterone, the (+) and (−) isomers differ little in potency. In man, metyrapone is metabolized to racemic metyrapol.

2-Methyl-1,2-di-3-pyridyl-1-propanone (**1**) (metyrapone) is used as a diagnostic tool for determining residual pituitary function in patients with hypopituitarism and, also, to evaluate a patient's ability to withstand surgery and other stresses.[1,2] Because metyrapone inhibits the adrenal cortex by inhibiting steroidal 11β-hydroxylation we added it to a fermentation to reduce the possibility of 11β-hydroxylation of a steroid by the microorganism *Botryodiplodia theobromae* Pat. The metyrapone was transformed by the organism. In subsequent experiments, in which no steroid was added, metyrapone was converted in good yield into a single more polar product, which proved to be the (−) isomer of the related racemic alcohol **2**.



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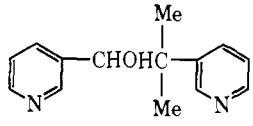

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Reduction of ketones to alcohols by microorganisms is known to be achieved with varying degrees of stereospecificity.[3] To determine the degree of specificity of the microbiological reduction of **1**, (−)-**2** ($[\alpha]^{21}D$ −41.5°) was converted into the acid salt of (−)-*O,O*-di-*p*-toluoyltartaric acid, which was then crystallized from MeOH. The rotation of the salt was not changed, and conversion to the base (−)-**2** gave material of $[\alpha]^{21}D$ −42.3°, a barely significant change from that of the microbiological product. The above procedure should completely resolve an already partially resolved material, provided that the chosen acid is a good resolving acid. This was shown to be so by reducing **1** with $NaBH_4$ to give racemic **2**, and then resolving it chemically with (+)-*O,O*-di-*p*-toluoyltartaric acid to give (+)-**2**, $[\alpha]^{21}D$ +42.7°. Thus the resolving acid was suitable, and the microbiological reduction was ~99% stereospecific in favor of the (−) isomer of **2**. It is assumed that no (+) isomer is selectively metabolized further. When the reduction of **1** by $NaBH_4$ was carried out without including an acid treatment in the work-up, the dihydrogen borate ester of (±)-**2** was obtained.

The alcohol **2** and its glucuronide are considered by Sprunt, *et al.*, to be metabolites of metyrapone in man.[4] The alcohol is also obtained by incubating metyrapone with rat adrenal quarters or liver tissue *in vitro*.[5] The authors made no comment about the optical activity of the product. Through the kindness of Dr. J. G. Sprunt, University of Dundee, Dundee, Scotland, we have been able to check this point for man. He supplied an extract of urines of patients dosed with metyrapone. The extract had been prepared by incubating the urines for 48 hr with crude β-glucuronidase obtained from limpets, at 37° and pH 5.5. A CH_2Cl_2 extract was obtained and concentrated at 37° *in vacuo*. We purified the crude metyrapol by the method used for the microbiologically produced material. It had $[\alpha]^{21}D$ ± 0° and mmp 100°, the same as that of racemic metyrapol. It is known that mammalian metabolism of ketones gives glucuronides of secondary alcohols, the alcohol moiety of which may or may not be optically active, depending upon the structure of the substrate.[6] It is reasonable to expect that no chemical racemization would have occurred during incubation at pH 5.5 with β-glucuronidase because the fermentation, which was carried out at pH 5.5–6.0 for 4 days, gave optically pure material. Nevertheless it seemed desirable to exclude the possibility of racemization under conditions of low pH. An aq solution of (+)-metyrapol at pH 1 (dil HCl) was kept at 37° for 48 hr. The observed rotation (0.29°) did not change during that time.

Mr. I. R. Hainsworth and Dr. J. K. Grant, University of Glasgow, have kindly made a preliminary examination of the ability of the (+) and (−) isomers of metyrapol to inhibit the hydroxylation of desoxycorticosterone to corticosterone by rat adrenals homogenized in sucrose solution. The incubations were for 1.5 hr at 37° in medium containing KCl–Tris buffer, pH 7.4–$MgSO_4$–NADP–glucose 6-phosphate–glucose 6-phosphate dehydrogenase, and [^{3}H]desoxycorticosterone. At 500 μg/incubation flask (5 ml of medium) the (+) and (−) isomers blocked the hydroxylation to the extent of 67 and 87%, respectively. The per cent conversion in the control was 30. Thus the isomers differ little in potency.

Experimental Section

Reduction of 2-Methyl-1,2-di-3-pyridyl-1-propanone (1) by *Botryodiplodia theobromae* Pat.—The organism was grown in a nutrient soln contg cerelose (dextrose monohydrate) (3%), ammonium tartrate (0.75%), KH_2PO_4 (0.2%), $MgSO_4 \cdot 7H_2O$ (0.05%), minor elements concentrate (0.1%),[7] and yeast ext (0.1%), adjusted to pH 5.5 before sterilization. Flasks contg

(1) J. J. Chart and H. Sheppard, *J. Med. Pharm. Chem.*, **1**, 407 (1959).
(2) W. L. Bencze and M. J. Allen, U. S. Patent 2,923,710 (1960).
(3) V. Prelog, *Ciba Found. Study Group [Pap.]*, **2**, 79 (1959).
(4) J. G. Sprunt, M. C. K. Browning, and D. M. Hannah, *Mem. Soc. Endocrinol.*, **17**, 198 (1968).
(5) I. Kraulis, H. Traikov, M. P. Li, C. P. Lantos, and M. K. Birmingham, *Can. J. Biochem.*, **46**, 463 (1968).
(6) T. H. Elliot, J. S. Robertson, and R. T. Williams, *Biochem. J.*, **100**, 393 (1966).
(7) P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, **29**, 173 (1946).

sterilized nutrient soln (200 ml) were inoculated with *B. theobromae* Pat.[8] and then incubated on a rotary shaker at 25°. After 3 days **1** (50 mg) in Me_2CO (1 ml) was added to each flask. Four days later the contents of 50 flasks (pH 6) were combined, adjusted to pH 2 with concd HCl (40 ml), shaken with EtOAc (3 l.), and then filtered through Celite to remove mycelium. The filtrate was sepd and the aq layer was washed with EtOAc (2 l.). The aq layer was adjusted to pH 11 with NaOH (8 *N*) and then extd 3 times with EtOAc (2 l. each time). The combined exts were washed twice with brine (500 ml each time), dried ($MgSO_4$), and then evapd to a gum (2.35 g). Tlc (silica gel GF; Et_2NH–EtOAc–C_6H_6, 5:77.5:17.5) showed a new product, R_f 0.15, containing a trace of starting material, R_f 0.38, visible at 254 mμ. This gum was chromatographed on Al_2O_3 (50 g, Grade III, neutral) and eluted with petr ether (bp 60–80°) contg increasing amts of C_6H_6, and then C_6H_6 contg increasing amounts of $CHCl_3$. The (−) isomer of **2** (2.2 g) was eluted in the range petr ether (60–80°)–C_6H_6 (4:1) to C_6H_6–$CHCl_3$ (9:1): mp 107–108° from EtOAc–petr ether (bp 60–80°), $[\alpha]^{21}D$ − 41.5° (*c* 1.1, EtOH); τ ($CDCl_3$) 5.38 (singlet, CHOH, 1); no molecular ion, *m/e* 121 $[C_5H_4N{\cdot}CH(CH_3)_2]^+$, 108 $[C_5H_4{\cdot}CHOH]^+$. *Anal.* ($C_{14}H_{16}N_2O$) C, H, N.

Check on Optical Purity of (−)-2-Methyl-1,2-di-(3-pyridyl)-1-propanol.—A soln of (−)-**2** (1.035 g, 0.0045 mole) and (−)-*O,O*-di-*p*-toluoyltartaric acid (1.72 g, 0.0045 mole) in MeOH (35 ml) at 50° was cooled slowly to room temp. The crystals which sepd were isolated, mp 155–156°, $[\alpha]^{21}D$ −109.1° (*c* 0.99, MeOH), and recrystd 3 times from MeOH to give (−)-**2** hydrogen (−)-*O,O*-di-*p*-toluoyltartrate hemihydrate (950 mg): mp 155–156°; $[\alpha]^{21}D$ −109.1° (*c* 0.99, MeOH). *Anal.* ($C_{34}H_{34}H_2O_9{\cdot}0.5H_2O$) C, H, N. (−)-**2**-Hydrogen (−)-*O,O*-di-*p*-toluoyltartrate hemihydrate (890 mg) was shaken with EtOAc (50 ml) and NaOH (0.5 *N*, 25 ml). The EtOAc extract gave (−)-**2** free base, mp 108–9° from EtOAc, $[\alpha]^{21}D$ −42.3° (*c* 0.99, EtOH).

(+)-**2-Methyl-1,2-di-(3-pyridyl)-1-propanol** (**2**).—A soln of racemic **2**, mp 100° (1.7 g, 0.0075 mole), and (+)-*O,O*-di-*p*-toluoyltartaric acid (2.8 g, 0.0073 mole) in MeOH (50 ml) at 50° was cooled slowly to room temp. The solid which sepd was recrystd 6 times from MeOH to give (+)-**2** hydrogen (+)-*O,O*-di-*p*-toluoyltartrate hemihydrate (1.3 g, 1st and 2nd crops) of constant rotation: mp 155–156°; $[\alpha]D$ + 108.6° (*c* 1.0, MeOH). *Anal.* ($C_{34}H_{34}N_2O_9{\cdot}0.5H_2O$) C, H, N. This salt gave (+)-**2** free base: mp 107–8°; $[\alpha]^{21}D$ +42.7° (*c* 1.1, EtOH). *Anal.* ($C_{14}H_{16}N_2O$) C, H, N.

1,2-Dipyrid-3-yl-2-methylpropyl Dihydrogen Borate (**3**). $NaBH_4$ (1 g) was added during 3 hr to a stirred soln of 2-methyl-1,2-di-3-pyridyl-1-propanone (2 g) in MeOH (30 ml) at 0°, and then kept for 2 hr. MeOH (15 ml) was removed *in vacuo*, brine (15 ml) was added, and the mixt was extd with EtOAc. The ext gave **3**, mp 192°, mass spectrum identical with that of (−)-**1**, τ (DMSO-d_6) 4.57 (broad singlet exchanged by D_2O, OH), 5.12 (singlet, CHO, 1). *Anal.* ($C_{14}H_{17}BN_2O_3$) H, N; C: calcd, 61.8; found 62.3.

Isolation of 2-Methyl-1,2-di-(3-pyridyl)-1-propanol from Urine Extract.—The crude ext (2.4 g) supplied by Dr. Sprunt was dissolved in H_2O (50 ml) and EtOAc (50 ml) and then the pH was adjusted to 2.0 with concd HCl. The mixt was shaken and then the aq acid layer was sepd, washed with EtOAc (50 ml), adjusted to pH 11 with NaOH (8 *N*), and then extd 3 times with EtOAc (100 ml each time). The combined exts were washed twice with brine (50 ml each time), dried ($MgSO_4$), and then evapd to a gum (1.18 g). This was chromatographed on Al_2O_3 as described above. The pure product was eluted in the range $CHCl_3$–C_6H_6 (1:19 to 1:3), (0.65 g), mmp 100° from EtOAc–petr ether (bp 60–80°), $[\alpha]^{21}D$ ± 0° (*c* 1.04, EtOH).

(8) Imperial Chemical Industries Ltd., A.C.C. 3121, kindly supplied by Royal Netherlands Fermentation Industries Ltd., Delft.

Stereochemical Studies on Medicinal Agents. 9.[1,2] Bicyclic Bases.[3] Synthesis and Biological Activities of Epimeric Quaternary Derivatives of 2-Oxa-5-azabicyclo[2.2.1]heptane[4]

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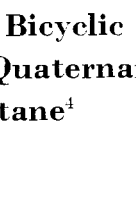
Optically active *N*-Me-*N*-benzyl quaternary derivatives of (1*S*,2*S*)-2-oxa-5-azabicyclo[2.2.1]heptane were synthesized in order to investigate the effect of an asymmetric quaternary N on anticholinergic activity. Nmr studies indicate that the N-substituted bicyclic system undergoes highly stereoselective quaternization. Configurations have been tentatively assigned to the N epimers. The *exo*-5-methyl-*endo*-5-benzyl and *exo*-5-benzyl-*endo*-5-methyl N epimers possess comparable antagonistic activities on the guinea pig ileum. The possible implications of the biological data are discussed.

Although the chiralities of ligands at cholinergic receptors have been investigated extensively,[5] little is known about the influence of an enantiomeric quaternary N on anticholinergic potency. Such information might complement existing data and provide a more coherent view of the interaction of anticholinergic ligands with cholinergic receptors.

Our approach to investigating this problem was to utilize the 2-oxa-5-azabicyclo[2.2.1]heptane system (**1**) as a probe, since endo–exo isomerism about the quaternary N in optically active **1** gives an enantiomeric N atom. Substituents (R, R′) not favorable for agonist activity would be expected to give antagonist, partial agonist, or inactive compounds.



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Chemistry.—The bicyclic intermediate **2** for the preparation of the desired compounds has been reported recently.[3] The absolute configuration of this compound is as depicted, since it was prepared from hydroxy-L-proline. Reduction of **2** with LAH failed to give optimal yields of the desired benzyl derivative **3** due to the

(1) We gratefully acknowledge support of this work by Public Health Service Grant GM 09402.

(2) Part VIII of this series: P. S. Portoghese and D. A. Williams, *J. Med. Chem.*, **13**, 626 (1970).

(3) Previous paper: P. S. Portoghese and J. G. Turcotte, *Tetrahedron*, in press.

(4) Presented in part at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, Abstract P-17.

(5) P. S. Portoghese, *Annu. Rev. Pharmacol.*, **10**, 51 (1970), and ref cited therein.