EXPERIENTIA

Vol. 31 – Fasc. 9 Pag. 1001 – 1112 15. 9. 1975

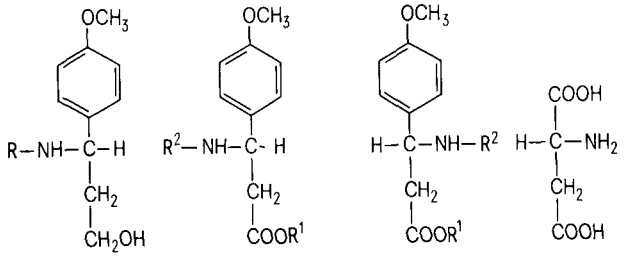
# SPECIALIA



## (*S*)-3-*p*-Methoxyphenyl-3-acetylaminopropan-1-ol, a New Metabolite of an Actinomycete[1]

In the course of the isolation of new antibiotics[2] from cultures of *Streptomyces michiganensis* CORBAZ et al., strain Tü 1074, an antibiotically inactive crystalline substance was obtained from side fractions during chromatography. Since the new compound is of some interest in connection with the metabolism of aromatic amino acids, we briefly report on its isolation, structure determination and synthesis.

*Streptomyces michiganensis* Tü 1074, isolated from a soil of El Djem (Tunesia), was fermented on a soybean meal – mannitol nutrient medium in submerse culture during 4 days. The broth was filtered and the filtrate extracted 3 times with chloroform. The concentrated extract was separated by chromatography on alumina (activity III, chloroform as the eluent) into 2 antibiotically active and an inactive crystalline fraction. The latter was rechromatographed on silica gel and recrystallized from chloroform – ether petroleum ether forming colorless needles, m.p. 124–124.5°; $[\alpha]_D = -151$. (c = 0.41, chloroform); yield 5 mg/l of culture fluid.



I R = $CH_3CO$ IIa $R^1 = R^2 = H$ II b V
VII R = H IIIa $R^1 = H, R^2 = CHO$ III b
IVa $R^1 = (R)$-sec.butyl IV b
$R^2 = CF_3CO$
VI $R^1 = CH_3, R^2 = H \cdot HCl$

The compound was neutral and gave no colour reaction with ninhydrin. In thin-layer chromatography on silica gel (Merck $F_{254}$, chloroform-methanol 9:1; $H_2SO_4$ spray) it gave a single spot, Rf 0.25. From elemental analysis (sample sublimed at 110°, 0.01 mm Hg) and the mass spectrum ($M^+$ 223) the molecular formula $C_{12}H_{17}NO_3$ was deduced. The UV-spectrum with $\lambda_{max}$ (log $\varepsilon$) 227 (4.06), 277 (3.19) and 283 nm (3.12) indicated the presence of an aromatic ring. The IR-spectrum (KBr) showed the characteristic absorption bands of a secondary amide (1635, 1545 $cm^{-1}$, whereas the region around 1700 $cm^{-1}$ is free of absorption bands.

In the NMR-spectrum the most prominent signals are: 2.03 (*s*, 3 H; $CH_3$-CO), 3.81 (*s*, 3 H; $CH_3O$-Ar), 6.87 and 7.21 (*AA'BB'*, 4 H; *p*-disubstituted benzene ring). A broad doublet at 6.28 ppm (1 H) is rapidly exchanged by trifluoroacetic acid, but not by $D_2O$ and is, therefore, the signal of an N-H (amide). A multiplet (min. 6 peaks; 1 H) near 5.1 ppm remains unchanged after exchange with $D_2O$, but is transformed into a 4 peak signal upon addition of trifluoroacetic acid ($J_1$ = 9 Hz, $J_2$ = 5 Hz) and is assigned to a proton adjacent to the acetamido group. Because of the high chemical shift of this signal, the CH-NH-Ac group must be attached to the benzene ring. A broad signal at 3.6 ppm (3 H) is converted to a 2 proton multiplet after exchange with $D_2O$ and is assigned to the 3 protons of a $CH_2OH$ group. The remaining signal is a broad multiplet (2 H) at 1.95 ppm ($CH_2$), partly covered by the acetyl singlet. Irradiation at 1.95 ppm (after exchange with trifluoroacetic acid) changed the signals at 3.6 and 5.1 ppm simultaneously to singlets. The only structure in agreement with all spectral data is that of 3-*p*-methoxyphenyl-3-acetamidopropan-1-ol (I).

Compound (I) gave an oily acetyl derivative with acetic anhydride and pyridine at room temperature. The product showed an additional acetyl signal at 1.99 ppm. The two proton multiplet, originally at 3.6 ppm, is shifted to 4.07 ppm, the other singals showing only minor changes. The NMR-spectrum and an IR-band at 1730 $cm^{-1}$ confirm that a primary alcoholic group was esterified.

The sense of chirality was determined by a synthesis of (I), starting from an optically active compound with known configuration. Racemic $\beta$-tyrosine methyl ether (II) was synthesized according to BIRKHOFER and KACHEL[3]. Its N-formyl derivative (III), prepared in the usual manner[4], could be partly resolved into enantiomers by repeated recrystallization of the (–)-cinchonidin salt from ethanol-ether. The most active salt obtainable by this method gave, by rinsing through a column of Dowex 50 × 8, an N-formyl-amino acid with $[\alpha]_D = -82°$ (c = 0.45, methanol), which proved to be ca. 60% optically

[1] Metabolic products of microorganisms; 148th communication. Preceding paper see: A. GERHARD, R. MUNTWYLER and W. KELLER-SCHIERLEIN, Helv. chim. acta, *58*, 1323 (1975).

[2] To be published in a forthcoming paper.

[3] L. BIRKHOFER and H. KACHEL in *Methoden der organischen Chemie* (Ed. HOUBEN-WEYL; Georg Thieme Verlag, Stuttgart 1958), vol. 11/2, p. 497.

[4] E. FISCHER and O. WARBURG, Ber. dt. chem. Ges. *38*, 3997 (1905).

pure by GLC of the derivative (IV)[5] (polypropylene glycol capillary column, 150°, helium stream). However, this partly resolved product could be further purified by recrystallization from acetone-ether. The purified product, (–)-N-formyl-β-tyrosine methyl ether, had m.p. 158–161° (decomp.) and $[\alpha]_D = -125°$ (c = 0.52, methanol); the optical purity was at least 91%, determined by GLC of (IVa). From the mother liquor of the cinchonidine salt (or from the less soluble diastereomer of the brucine salts), the partly purified enantiomer IIIb of IIIa was obtained and further purified to at least 95% optical purity: m.p. 158–161° (decomp.) $[\alpha]_D = +130°$ (c = 0.54, methanol).

The dextrorotatory N-formylamino acid (IIIb) (202 mg) was ozonized in 30 ml of 5% aqueous formic acid according to CORRODI and HARDEGGER[6] during 24 h, and the ozonide cleaved by the addition of 2 ml of 30% hydrogen peroxide in 2 ml of formic acid (*20 h, 20°*). From the evaporated reaction mixture, a 24% yield of of D-aspartic acid (V), $[\alpha]_D = -27{,}1°$ (c = 1,77 in 5 *N* HCl) was obtained by recrystallization from water, and identified by the IR-spectrum and by TLC. The dextrorotatory formylamino acid (IIIb) has therefore the (*R*)-chirality.

The levorotatory (*S*)-formyl derivative (IIIa) (91% optical purity) was transformed to the oily methyl ester hydrochloride (VI) by simultaneous deformylation and esterification (abs. methanol saturated with dry HCl; *24 h, 20°*). The ester was reduced with excess lithium aluminium hydride in abs. dioxane (1 h, reflux) to the liquid amino alcohol (VII). The crude product was isolated from the reaction mixture by decomposition with water and extraction with chloroform. The mixture contained a major component (Rf 0.5, TLC with butanol – acetic acid – water 4:1:1; ninhydrin positive) and several unidentified minor impurities (Rf 0.16 up to 0.9). The IR-spectrum showed no absorption in the carbonyl region.

Selective N-acetylation of 85 mg of crude (VII) was carried out with 1 ml of acetic anhydride in 5 ml of methanol (5 h, 20°) to yield the final product (I), which was purified by chromatography on silica gel, recrystallization and sublimation at 0.01 mm Hg and 105–110°; over all yield 15% from IIIa. The m.p. and mixed m.p. were 124–124.5°; $[\alpha]_D = -149°$ (c = 0.47, chloroform). The Rf value (TLC), IR- and NMR-spectrum, showed no difference from those of the natural product, which therefore has the (*S*)-chirality.

Racemic I, prepared in the same manner from racemic II, had m.p. 108°; the NMR-spectrum and IR-spectrum in chloroform were identical with those of the optically active compound. However, the IR-spectra in KBr showed marked differences in the finger-print region.

*Summary.* (*S*)-3-*p*-methoxyphenyl-3-acetamidopropan-1-ol was isolated from cultures of an actinomycete (*Streptomyces michiganensis*). Its structural determination by spectroscopic means and its synthesis are described.

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*14 May 1975.*

[5] R. CHARLES-SIGLER and E. GIL-AV, Tetrahedron Lett. *1966*, 4231. The (*R*), (+)-sec. butyl ester of the N-trifluoroacetyl (*S*)-amino acid showed $R_t$ 40.8 min, the diastereomeric (*R*), (*R*)-compound 39.4 min.

[6] H. CORRODI and E. HARDEGGER, Helv. chim. acta *38*, 2038 (1955).

## The Absolute Configuration of the Enantiomers of Glutethimide and Aminoglutethimide

Aminoglutethimide (Elipten® CIBA) was originally introduced as an anticonvulsant for the treatment of epilepsy. It was subsequently withdrawn because of inhibitory effects on adrenal function[1]. Recently these adrenal effects have been suggested to be of utility in the treatment of metastatic breast cancer[2,3]. Because aminoglutethimide also inhibits ovarian secretion of progesterone, the potential abortifacient properties of aminoglutethimide have been investigated[4–6]. Further chemical studies have also been reported recently[7,8].

In view of this renewed interest in aminoglutethimide, we decided to resolve it and explore the biological properties of its antipodes I and II (levo- and dextro-rotatory, respectively). We postulated that the steroid synthesis inhibiting properties might reside in one antipode and the potency could therefore be enhanced by resolution.

The antipodes were tested in parallel with racemic aminoglutethimide for their effects on adrenal and ovarian steroid secretion in rats. Changes in corticosterone secretion were measured in adrenal vein blood obtained before and after i.v. injection of aminoglutethimide or its antipodes into anesthetized rats. The dextrorotatory antipode II was 2 or 3 times more potent an inhibitor than the racemate, while levorotatory antipode I had very little activity at dose levels 10-fold higher. In gonadotrophin-primed immature female rats[9], antipode II injected i.v. reduced mean plasma progesterone levels by more than 78% and accounted for most of the activity in the racemate.

Resolution of aminoglutethimide, therefore, provided a compound (antipode II) with essentially all of the steroid synthesis inhibiting activity of the racemate.

Glutethimide (Doriden® CIBA and USV) had been previously resolved[10], and biological studies indicated

[1] A. M. CAMACHO, R. CASH, A. J. BROUGH and R. S. WILROY, J. Pediat. *6*, 852 (1966).
[2] C. T. GRIFFITHS, T. C. HALL, Z. SABA, J. J. BARLOW and H. B. NEVIAMY, Cancer *32*, 31 (1973).
[3] R. J. SANTEN, A. LIPTON and J. KENDALL, J. Am. med. Ass. *230*, 1661 (1974).
[4] S. R. GLASSER, R. C. NORTHCUTT, F. CHYTIL and C. A. STROTT, Endocrinology *90*, 1363 (1972).
[5] H. R. BEHRMAN and R. O. GREEP, Contraception *4*, 229 (1971).
[6] B. G. STEINETZ, W. H. NIEMANN, T. GIANNINA, D. DUNCAN and J. J. CHART, Proc. Soc. exp. Biol. Med. *148*, 790 (1975).
[7] R. PAUL, R. P. WILLIAMS and E. COHEN, J. med. Chem. *17*, 539 (1974).
[8] E. E. SMISSMAN, P. J. WIRTH and D. R. GLYNN, J. org. Chem. *40*, 281 (1975).
[9] O. D. SHERWOOD, M. L. BIRKHIMER and D. G. PARKES, Endocrinology *93*, 723 (1973).
[10] S. KUKOLJA, D. GRGURIC and L. LOPINA, Croat. chim. Acta *33*, 41 (1961).
[11] K. SCHMID, W. RIESS and H. KEBERLE, Isotopes, exp. Pharmac. *1965*, 383.