M. Hoyte, J. F. Klopper, S. S. Lin, and A. P. Wolf, *J. Nucl. Med.*, 13, 713 (1972).
(11) J. S. Fowler, A. N. Ansari, H. L. Atkins, P. R. Bradley-Moore, R. R. MacGregor, and A. P. Wolf, *J. Nucl. Med.*, 14, 867 (1973).
(12) J. Häggendahl, *Scand. J. Clin. Lab. Invest.*, 14, 537 (1962).

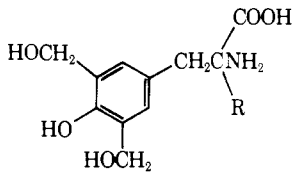
Saligenin Analogs of *l*-Dopa and *dl*-α-Methyl-Dopa

Michael Atkinson, David Hartley,* Lawrence H. C. Lunts, and Alexander C. Ritchie

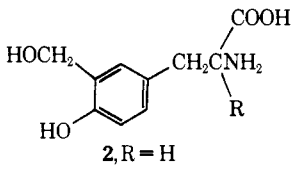
Chemistry Department, Allen and Hanburys Research Ltd., Ware, Hertfordshire, England. *Received July 12, 1973*

We have described[1] some saligenin derivatives that retain the adrenergic activity of the corresponding catechols and the discovery of a long-acting and highly selective bronchodilator [salbutamol (Ventolin)]. It seemed worthwhile to modify other biologically active catechols in a similar fashion and to study their properties. We now wish to report the preparation of saligenin analogs of Dopa and α-methyl-Dopa as potential drugs for the treatment of Parkinson's disease and hypertension, respectively.

These syntheses were achieved by hydroxymethylation of the *N*-benzyloxycarbonyl derivatives of the corresponding tyrosines, using HCHO and NaOH at room temperature. The protecting group was removed by catalytic hydrogenolysis, using Pd black in the presence of NH_4OH to conserve the saligenin function. The products from reactions with 1 molar equiv of HCHO were shown by thin-layer and paper chromatography to be mixtures containing unchanged starting materials and bishydroxymethylated compounds **1** in addition to the saligenins **2** or **3**. The bishydroxymethylated compounds were identified by singlet peaks in their nmr spectra (D_2O–NaOH) at δ 4.62 (4 H, CH_2OH) and 6.97 (2 aromatic protons). Even when the amount of HCHO was reduced to between 0.8 and 0.2 molar equiv there was always a preponderance of the bishydroxymethylated product **1** over the desired mono derivative **2** or **3**. This problem was overcome by adding $Na_2B_4O_7$ to chelate with the saligenin moiety of the initial product. Thus, by binding the phenolic group, further nuclear substitution is prevented and the required amino acids **2** and **3** were obtained in overall yields of greater than 25%.



1, R = H or Me



2, R = H
3, R = Me

The hydroxymethylation step required 14–20 days at room temperature and was difficult to force to completion. Hydrogenolysis of the product from reactions using shorter times always gave some unchanged *l*-tyrosine or *dl*-α-methyltyrosine, although these materials could be removed by preferential crystallization from H_2O.

Neither compound *l*-**2** nor *dl*-**3** lowered the systolic blood pressure of the DOCA hypertensive rat[2] in doses of 50–200 mg/kg given subcutaneously each day for 3 days, whereas *l*-α-methyl-Dopa produced a fall of 35 mm after a single dose of 20 mg/kg and of 60 mm after three doses of 20 mg/kg given over 2 days. Using the procedure described by Maj, *et al.*,[3] both *l*-Dopa and the analog **2** at doses of 250 mg/kg, ip, reversed the catalepsy induced in rats by ip administration of 0.5 mg/kg of haloperidol, a blocker of dopaminergic receptors. However, this was the only test that suggested biological similarity. In other tests in which *l*-Dopa is effective, such as the enhancement of circulatory behavior of rats with a unilateral lesion in the nigrostriatal system,[4] or the antagonism of tremor induced in rats by harmine,[5] the saligenin **2** was inactive in doses of 250 mg/kg, ip.

From these results it seems unlikely that the compounds would be of value in the treatment of hypertension or Parkinson's disease.

The absence of the desired activities in **2** and **3** may be related to their lack of significant interaction with Dopa decarboxylase. Thus, they did not inhibit kidney Dopa decarboxylase from the guinea pig or rat at a concentration of 1.54×10^{-3} *M* (ID_{50} of *dl*-α-methyl-Dopa is 7×10^{-6} *M*).[6] Neither were the compounds substrates (for the experimental procedure see ref 7) at the same concentration (K_m of Dopa is 1.7×10^{-3}). These *in vitro* results might also explain why no evidence could be found for depletion of levels of norepinephrine in mouse hearts,[8] as little of the corresponding dopamines would be formed to displace norepinephrine from its stores.

Experimental Section

Melting points were determined on a Townson and Mercer melting point apparatus and the microanalyses on an F & M 185 C, H, and N analyzer. Where analyses are indicated by the symbols of the elements, analytical values were within ±0.4% of the calculated values. Compounds gave satisfactory uv, ir, and nmr spectral data obtained respectively on Perkin-Elmer Model 137, Unicam SP 100, and Varian Associates A-60A spectrometers. Paper and tlc chromatograms were run on Whatman No. 1 paper (BuOH–AcOH–H_2O, 60:15:25) and SiO_2 (EtOAc–MeOH, 1:1), respectively, and the products identified by staining blue with 1% $FeCl_3$ solution.

l-*N*-**Benzyloxycarbonyl-3-(hydroxymethyl)tyrosine.** A solution of $Na_2B_4O_7$ (14 g) in H_2O (200 ml) and 36% HCHO (10 ml, 0.1 mol) was added to *l*-*N*-benzyloxycarbonyltyrosine (10 g, 0.03 mol) in 1 *N* NaOH (60 ml). After 14 days at room temperature no starting material could be detected by tlc. The pH was adjusted to between 1 and 2 with 1 *N* HCl to deposit a white oil which was extracted into EtOAc. The extracts were washed with brine, dried ($MgSO_4$), and evaporated to give a clear oil which solidified on standing: 9 g; mp 125–129°. Two recrystallizations from EtOAc gave *l*-*N*-benzyloxycarbonyl-3-(hydroxymethyl)tyrosine as a white powder: 6.9 g (63%); mp 131.5–133°; $[\alpha]^{24.5}D$ +10.4° (*c* 0.75, AcOH). *Anal.* ($C_{18}H_{19}NO_6$) C, H, N.

l-**3-(Hydroxymethyl)tyrosine** (**2**). A solution of *l*-*N*-benzyloxycarbonyl-3-(hydroxymethyl)tyrosine (2.2 g, 0.0064 mol) in EtOH (30 ml) and 1 *N* NH_4OH (7 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of Pd black (0.5 g). Uptake of H_2 (0.0056 mol) ceased after 1 hr. The catalyst and solvent were removed to leave the amino acid as a white powder: 1.2 g (88%); mp >300°; $[\alpha]^{24}D$ −9.2° (*c* 0.38, 0.1 *N* HCl) *Anal.* ($C_{10}H_{13}NO_4 \cdot 0.25H_2O$) C, H, N.

dl-**3-(Hydroxymethyl)-α-methyltyrosine** (**3**). *dl*-α-Methyltyrosine[9] reacted with benzyl chloroformate and NaOH by a standard procedure[10] to give *dl*-*N*-benzyloxycarbonyl-α-methyltyrosine in 65% yield: mp 183–184°. *Anal.* ($C_{18}H_{19}NO_5$) C, H, N. The latter (6 g, 0.018 mol) was hydroxymethylated in a manner similar to that described for the corresponding tyrosine derivative and the crude product hydrogenated to give *dl*-3-(hydroxymethyl)-α-methyltyrosine (**3**): 1.8 g (41%); mp >300°. *Anal.* ($C_{11}H_{15}NO_4$) C, H, N.

Acknowledgment. We thank our colleagues Dr. R. T. Brittain, Mr. L. E. Martin, and Dr. J. Hunt and their staff for pharmacological, biochemical, and analytical data, respectively, and Mr. M. T. Davies of BDH (Research), Ltd., London, for the optical rotations. We are especially grateful to Dr. R. J. Naylor of the University of Bradford for carrying out the pharmacological tests to assess the potential of some of the compounds in Parkinson's disease.

References

(1) D. T. Collin, D. Hartley, D. Jack, L. H. C. Lunts, J. C. Press, A. C. Ritchie, and P. Toon, *J. Med. Chem.*, 13, 674 (1970).
(2) R. Gross, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **232**, 161 (1957).
(3) J. Maj, Z. Kapturkiewicz, and J. Sarnek, *J. Pharm. Pharmacol.*, **24**, 735 (1972).
(4) U. Ungerstedt, *Acta Physiol. Scand., Suppl.*, **367**, 69 (1971).
(5) D. M. Kelly, Ph.D. Thesis, University of Bradford, 1973.
(6) A. Parulkar, A. Burger, and D. Aures, *J. Med. Chem.*, 9, 738 (1966).
(7) R. Ferrini and A. Glasser, *Biochem. Pharmacol.*, 13, 798 (1964).
(8) J. W. Daly, C. R. Creveling, and B. Witkop, *J. Med. Chem.*, 9, 273 (1966).
(9) W. S. Saari, *J. Org. Chem.*, **32**, 4074 (1967).
(10) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

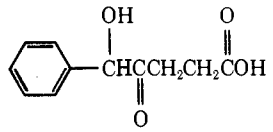
5-Hydroxy-5-phenyllevulinic Acid. A Novel Microbial Metabolite with Analgesic Activity

E. R. Wetzel, A. C. Osterberg, and D. B. Borders*

Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965.

Received August 2, 1973

During a search for microbial metabolites with pharmacological activity, we investigated compounds produced by a fungus belonging to the *Tubercularia* species, designated in our laboratories as Z1497. When Z1497 was grown on a complex medium, the culture filtrate contained a major metabolite which could be extracted with 1-butanol. This compound (1) was readily purified by chromatography on silica gel and crystallization from benzene which yielded colorless, long-needled rosettes, mp 86–87°, $[\alpha]^{25}D$ +155°.



1

The molecular formula, $C_{11}H_{12}O_4$, was deduced from the mass spectrum (parent ion *m/e* 208), elemental analyses, and the nmr spectrum (12 protons). A phenyl group was suggested by absorption near λ_{max} 260 nm with characteristic fine splitting indicating an isolated benzene chromophore. The mass spectrum had peaks at *m/e* 77, 78, and 79, indicative of a monoalkylbenzene-type structure, which is substantiated by a 5-proton singlet (δ 7.39, overlapped by two ionizable protons) in the nmr spectrum and by strong absorption at 697 and 759 cm^{-1}. The presence of a COOH group was shown by the ir spectrum (OH at 3500–2500 cm^{-1}, -C=O at 1715 cm^{-1}) and the reaction of the compound with aqueous sodium bicarbonate to evolve carbon dioxide. A positive reaction with 2,4-dinitrophenylhydrazine, the lack of an aldehyde proton in the nmr spectrum, and a shoulder near 1725 cm^{-1} indicated the presence of a keto group. There is a 4-proton absorption at δ 2.62 attributed to two almost equivalent methylene groups. The chemical shift and general appearance of this signal are very similar to those of the methylene groups of levulinic acid[1] which suggests the following unit in the Z1497 metabolite: $-C(=O)CH_2CH_2C(=O)OH$. The remaining structural feature is a >CHOH group which would be between the keto group and the phenyl ring. The base peak (*m/e* 107) of the mass spectrum is explained by fragmentation to give the ion $[C_6H_5GHOH]^+$. This structural unit also accounts for the nonionizable proton at δ 5.18 and one of the ionizable protons overlapping the absorption of the aromatic protons. The resulting novel structure (1) for the Z1497 metabolite has an asymmetric center which is consistent with the observed optical activity.

Pharmacology. Compound 1 was active by the mouse writhing syndrome.[2] In a low intensity rat tail-radiant heat experiment,[3,4] an intraperitoneal dose of 1 at 50 mg/kg induced a 75% rise in pain threshold whereas aspirin at the same dose level was inactive. By the inflamed rat paw pain test[5] the metabolite was slightly better or at least equivalent to aspirin.

The biological activity of 1 is of particular interest since few microbial metabolites with analgesic activity have been discovered.[6–8]

Experimental Section

Melting points are uncorrected. Nmr, ir, mass, and uv spectra were obtained on Varian A-60A and A-60D, Perkin-Elmer Model 21, AEI MS-9, and Cary Model 11 M spectrometers, respectively. Chemical shifts are reported as δ with tetramethylsilane as an internal reference.

Fermentation. Culture Z1497 was grown in 300-l. fermentors under standard conditions of aeration and agitation on a medium containing the following ingredients (grams per liter): glucose, 10; molasses, 20; tryptone, 10. The course of the fermentation was followed by extracting time samples of the culture filtrate with 1-butanol and analyzing the extracts by tlc on silica gel F-254 plates (Brinkmann Instruments, Inc.) developed with the system 1-butanol saturated with water. The desired product (R_f 0.5) was detected with a dinitrophenylhydrazine spray.

Isolation. The harvested mash filtrate was extracted with 175 l. of 1-butanol and the extract was evaporated to a 5-l. aqueous concentrate. The concentrate was extracted with ethyl acetate and the resulting extract was evaporated to a syrup which was redissolved in chloroform.

The chloroform solution, 200 ml, was then charged on a silica gel column (6 × 40 cm, Davison Co., 60–200 mesh). The column was eluted with chloroform at a flow rate of approximately 12 ml/min.

The manner of pooling the column fractions was determined by drying small aliquots of each one and visibly comparing the solids. Concentration of the pooled fractions corresponding to 1000–4000 ml of elute yielded crystalline material on standing. Recrystallization from warm benzene yielded 6.3 g of the crystalline metabolite.

For analytical samples, the metabolite was recrystallized from warm benzene and dried at 56°: λ_{max} (MeOH) 210 nm (ε >5000), 260 (270), 284 (270); $[\alpha]^{25}D$ +155° (*c* 0.692, MeOH); nmr ($CDCl_3$) δ 7.39 (s, 7 H, includes 2 exchangeable H's), 5.18 (s, 1 H), 2.62 (m, 4 H); ir (KBr) 3420, 3150 b, 1725 sh, 1715, 1400, 1248, 1215, 1163, 1078, 1012, 853, 828, 759, and 697 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 208 (0.5), 180 (15), 107 (100), 105 (17), 101 (13), 79 (48), 51 (15), 28 (15), 18 (65).

Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.46; H, 5.76; mol wt, 208. Found: C, 63.84; H, 5.86; mol wt, 208 (mass spectrum).

Acknowledgment. We wish to thank Mr. L. Brancone and staff for microanalyses, Mr. W. Fulmor and staff for spectral analyses, Mr. A. J. Shay for the large-scale fermentation, and Dr. H. D. Tresner for the isolation and taxonomic study of the organism.

References

(1) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," Wiley, New York, N. Y., 1967, p 193.
(2) L. C. Hendershot and J. Forsaith, *J. Pharmacol. Exp. Ther.*, 125, 237 (1958).
(3) F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.*, 72, 74 (1941).
(4) W. D. Gray, A. C. Osterberg, and T. J. Scuto, *J. Pharmacol. Exp. Ther.*, 172, 154 (1970).
(5) L. O. Randall and J. J. Selitto, *Arch. Int. Pharmacodyn.*, 111, 409 (1957).
(6) L. H. Conover, *Advan. Chem. Ser.*, **No. 108**, 33 (1971).