Enzymatic Synthesis of (*S*)-(-)-3-(3,4-Dihydroxyphenyl)lactic Acid[1)]

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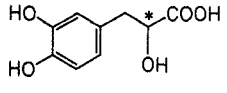
The first enzymatic synthesis of (*S*)-(-)-3-(3,4-dihydroxyphenyl)lactic acid, a DOPA metabolite and naturally occurring compound with a broad spectrum of pharmacological activities, is described. L-Hydroxyisocaproate dehydrogenase was used as enzyme.

Enzymatische Synthese von S-(-)-3-(3,4-Dihydroxyphenyl)-milchsäure

Die enzymatische Synthese von S-(-)-3-(3,4-Dihydroxyphenyl)-milchsäure, eines Dopa-Metaboliten sowie eines nativen Wirkstoffes mit einem breiten Spektrum pharmakologischer Wirkungen, unter Verwendung von L-Hydroxyisocaproat-Dehydrogenase wird erstmals beschrieben.

3-(3,4-dihydroxyphenyl)lactic acid **(1)** was first suggested by *Smith*[2)] to be a minor metabolite of 3,4-dihydroxyphenylalanine (DOPA) **(2)**, an amino acid formed during the biosynthesis of noradrenaline and adrenaline and widely used in its *levo*-form in the treatment of *Parkinson*'s disease. Its formation in meal worms following administration of adrenaline was reported much earlier[3)] and it has also been identified as a metabolite of safrole epoxide in rats and guinea pigs[4)]. Its *R*(+)-form has been identified as a water-soluble constituent of the roots of the Chinese medicinal herb *Salvia miltiorrhiza* (Dan Shen Su)[5)]. It has also been identified very recently in the Ceptidis rhizome[6)]. The pharmacological properties of **1** have been the subject of extensive investigations by Russian and Chinese workers. The following actions have been attributed to **1**: hypothermia and decrease in exploratory behaviour[7)]; competitive inhibition of aromatic amino acid pyridoxal decarboxylase[8, 9)]; enhancement of the central effects of L-DOPA[10)]; marked vasodilatory action on isolated swine coronary artery preparations[11)]; increase in the tolerance of mice to hypoxia[12)]; antagonization of the constrictor response to morphine and propanolol[13)] and, anticoagulation effects in vitro[14)]. Its actions have also been investigated to understand the underlying mechanisms in the pathogenesis of extrapyramidal disorders[15)].



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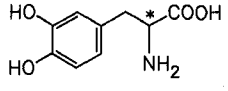

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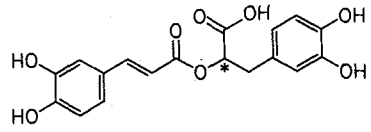
The synthesis of **1** was first claimed in a patent by *Nagoya* et al.[16)]. According to them, sodium 3-(3,4-dibenzyloxyphenyl)glycidate can be reduced catalytically by hydrogen to give the sodium salt in 82 % yield. *Xue* et al. have reported the synthesis of the sodium salt starting from 3,4-dihydroxybenzaldehyde in 37 % overall yield using the azlactone route and subsequent *Clemmensen* reduction[17)]. Both methods, however, lead to racem. **1**.

Probably the enantiomers of **1** might possess different pharmacological actions. Besides, the enantiomers could serve as starting materials for the synthesis of other naturally occurring polyphenolic acids such as rosmarinic acid **(3)**. Therefore, it was desirable to develop a new route to synthesize the enantiomers of **1** in good yields. Preliminary experiments using classical routes to 2-hydroxy acids such as oxidative deamination of the corresponding amino acid and the method employing reduction with Hantzsch esters were unsuccessful. Therefore, we investigated the enzymatic reduction of the corresponding 2-keto acid **4**.



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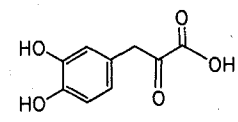

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Since the first enzymatic reduction of pyruvate to lactate using L-lactate dehydrogenase and $NADH_2$ was reported by *Meister* in 1950[18)], a wide range of 2-keto acids have been investigated using various oxido-reductases. Particularly in the last fifteen years, there has been a spurt in the use of enzymes in organic synthesis[19)]. Polyphenolic compounds, especially those containing ortho- or para-dihydroxyphenyl moieties, are a highly significant group of substrates suitable as targets for enzymatic reactions because they are easily susceptible to oxidation and the chemical synthesis using protective group techniques generally yield products only with considerable difficulty. Surprisingly, this group of substrates has been left largely ignored till now. In this paper, we wish to report the first enantioselective, enzymatic synthesis of (*S*)-(-)-**1**.

The substrate for the present investigation, 3-(3,4-dihydroxyphenyl)pyruvic acid **(4)**, was synthesized starting from DOPA according to *Steglich*[20)]. For the reduction, L-2-hydroxyisocaproate dehydrogenase (L-HicDH) was chosen since it was reported to be active in reducing a wider range of substrates compared to L-lactate dehydrogenase[21)]. This enzyme is also dependent on the cofactor $NADH_2$. From preliminary experiments it could be established by tlc that the hR_f value of the product formed was identical to that reported for **1**[22)]. In subsequent experiments, the cofactor could be successfully regenerated employing the well-established procedure using formate dehydrogenase (FDH) and ammonium formate[23)]. Apart from tlc experiments, the λmax of the product in the UV-spectrum at 279 nm coincides with that reported[24)]. In the mass spectrum of **1** $M^{+\cdot}$ appears expectedly at m/z = 198. The appearance of the base peak at m/z = 123, corresponding to the dihydroxybenzyl moiety, is in accordance with those reported in the literature for analogous compounds[25)].

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Discussion

The above experiments indicate a feasible method for the enzymatic synthesis of *S*-(–)-3-(3,4-dihydroxyphenyl)lactic acid **(1)** using L-HicDH. Since no protection of the phenolic OH-groups is essential, this method could prove to be viable for the large-scale synthesis of this class of compounds. Investigations for the synthesis of the *R*-(+)-enantiomer, methods employing other oxido-reductases as well as the synthesis of **1** in an enzyme membrane reactor using the well-established technique of continuous cofactor regeneration[27] will be reported shortly. The synthesis of **1** starting from DOPA itself using a multi-enzyme system (oxidative deamination to the keto-acid followed by reduction to the 2-hydroxy acid) is a further possibility.

Finally, the above synthesis raises hopes of a synthetic route to rosmarinic acid (2-caffeoyl-3-(3,4-dihydroxyphenyl))-D-lactic acid **(3)**, a compound undergoing extensive investigations for its anti-inflammatory properties. Although the structure of rosmarinic acid was first elucidated by *Scarpati* thirty years ago[28], its synthesis has always posed a formidable challenge.

Our thanks are due to Prof. *C. Wandrey*, Jülich, for providing the facilities to carry out the enzymatic experiments, to Prof. *M.-R. Kula* (now in Jülich) and her group at GBF, Braunschweig, for the enzymes, to Prof. *H. Kneifel*, Jülich, for the mass spectrum, and to Ms. *B. Reimann* for technical assistance.

Experimental Part

The substrate **4** was synthesized as mentioned earlier; m. p. 188–89 °C (Lit.[20] 189–90 °C). It was found to be a single compound by tlc. – The enzymes L-HicDH and FDH were gifts from GBF, Braunschweig, FRG. – $NADH_2$ was purchased from Boehringer Mannheim, FRG. Optical rotation: Perkin-Elmer Polarimeter (Model 241). – TLC: Polyamide 6 Flexible Sheets, J. T. Baker, Phillipsburg, USA, Avicel Cellulose F, E. Merck, Darmstadt, FRG and Kieselgel F_{254}, E. Merck using a 1 % solution of fluorescein in absol. ethanol as indicator. Short path column chromatography: microcrystalline cellulose (Avicel, E. Merck) with 0.001M H_2SO_4 as eluant. – Mass spectrum: Kratos MS 25 spectrometer, 70 eV.

S-(–)-3-(3,4-Dihydroxyphenyl)lactic acid **(1)**

3 (25 mmol/L), L-HicDH (5 U/mL) and $NADH_2$ (50 mmol/L) were dissolved in TRIS-maleate buffer pH 6.50 (trometamine 200 mmol/L; maleic acid 200 mmol/L; adjusted to pH 6.50 with NaOH) in an amber-colored glass container which was previously dried at 100 °C and cooled to room temp. in a stream of argon. The glass container was placed in a water bath maintained at 25 °C and its contents were stirred continuously. A stream of argon was bubbled through the solution. After 4 h, the flow of argon was stopped and the experiment was discontinued. A small sample was analysed by tlc, the results are summarized below:

1. Solvent system: chloroform/ethyl acetate/acetic acid (70/20/10) hR_f: 3–4 (Lit.[22]: 3)
2. Solvent system: benzene/2-butanone/methanol (60/20/20) hR_f: 18–20 (Lit.[22]: 20)

Using cellulose layers as described[26] and employing the solvent system 2-propanol/water (50/10), an hR_f value of 62–63 was obtained. With silica gel, the hR_f value was 51–52 when the solvent system n-butanol/acetic acid/water (63/10/27) was used.

In a further experiment, the procedure was repeated but the $NADH_2$ concentration was reduced to 1 mmol/L. FDH (5U/mL) and ammonium formate (25 mmol/L) were added to regenerate the cofactor continuously. The optical rotation of a sample removed from the reactant mixture and subjected to ultrafiltration was measured periodically. The experiment was discontinued when no further change in the optical rotation could be observed (ca. 16 h). The maximum optical rotation caused by the product was –0.180°. Ultrafiltration, concentration of the filtered product solution, column chromatography, extraction with ethyl acetate and, finally, removal of ethyl acetate led to **1**.

References

1 Taken in part from the Ph. D. thesis of N. N. Rao, Tübingen 1986 (Ref. H. J. Roth).
2 P. Smith, Nature *213*, 802 (1967).
3 R. I. Gregerman and G. Wald, J. Gen. Physiol. *35*, 489 (1952).
4 W. G. Stillwell, M. J. Carman, L. Bell and M. G. Horning, Drug Metab. Dispos. *2*, 489 (1974).
5 D.-C. Zhang, C.-L. Wu, H.-C. Liu, T.-C. Pan, C.-H. Yang and P.-T. Chang, Shang-hai Ti I I Hsueh Yuan Hsueh Pao *7* 384 (1980); C. A. *94*, 127201n (1981).
6 S. Yahara, M. Satoshiro, I. Nishioka, T. Nagasawa and H. Oura, Chem. Pharm. Bull. *33*, 527 (1985).
7 A. E. Uspenskii, Farmakol. Toksikol. (Moscow) *41*, 418 (1978); C. A. *89*, 157349f (1978).
8 A. E. Uspenskii, A. P. Skoldinov and V. T. Klimko, Khim.-Farm. Zh. *12*, 19 (1978); C. A. *89*, 173636c (1978).
9 A. E. Uspenskii and V. P. Nuzhnyi, Farmatsiya (Moscow) *31*, 64 (1982); C. A. *98*, 119174y (1983).
10 A. E. Uspenskii, N. S. Dabagov and A. P. Skoldinov, Byull. Eksp. Biol. Med. *86*, 444 (1978); C. A. *90*, 115067c (1979).
11 Z.-T. Dong and W.-D. Jiang, Shang-hai Ti I I Hsueh Yuan Hsueh Pao *8*, 79 (1981); C. A. *95*, 143854d (1981).
12 W. Chiang and Y. Wang, Yao Hsueh T'ung Pao *16*, 59 (1981); C. A. *95*, 143964q (1981).
13 Z. Dong and W. Jiang, Youxue Xuebao *17*, 226 (1982); C. A. *97*, 66002m (1982).
14 Y. Shi, Z. Liang and Y. Bu, Zhongyao Tongbao *11*, 432 (1986); C. A. *105*, 164702t (1986).
15 F. S. Messiha, Brain Res. Bull. *11*, 233 (1983).
16 German Offen. DE 2047839, 15. April 1971 (T. Nagoya, Y. Fujima and Y. Shimizu, Sankyo Chem. Industries Co. Ltd.); C. A. *75*, 19953h (1971).
17 F. Xue, H. Dai and L. Ding, Shang-hai Diyi Yixueyuan Xuebao *10*, 133 (1983); C. A. *99*, 212229a (1983).
18 A. Meister, J. Biol. Chem. *184*, 117 (1950).
19 See, for example, J. B. Jones, Tetrahedron *42*, 3351 (1986).
20 C. Klein, G. Schulz and W. Steglich, Liebigs Ann. Chem. 1638 (1983).
21 H. Schütte, W. Hummel and M.-R. Kula, Appl. Microbiol. Biotechnol. *19*, 167 (1984).
22 S. D. Harrison, Jr., P. Chiu and R. P. Maickel, J. Chromatogr. *85*, 151 (1973).
23 H. Schütte, J. Flossdorf, H. Sahm and M.-R. Kula, Eur. J. Biochem. *62*, 151 (1978).
24 Z. Qin, Z. Liang, H. He, C. Yang, B. Zhang, D. Zhang and J. Jin, Yaowu Fenxi Zazhi *3*, 214 (1983); C. A. *99*, 146175b (1983).
25 C. J. Kelley, R. C. Harruff and M. Carmack, J. Org. Chem. *40*, 1804 (1975).
26 M. Hanefeld and K. Herrmann, J. Chromatogr. *123*, 391 (1976).
27 U.S. Patent 4326031 of 20th April 1982 (to C. Wandrey, R. Wichmann, W. Leuchtenberger, M.-R. Kula and A. Bückmann).
28 M. L. Scarpati and G. Oriente, Ric. Sci. *28*, 2329 (1958).

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