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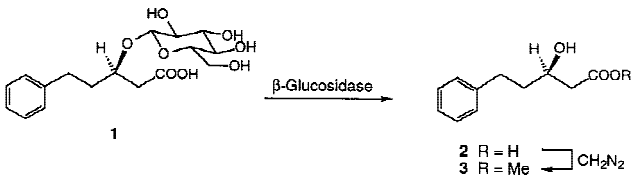
Determination of the Absolute Configuration of (–)-(3*R*)-*O*-β-D-Glucopyranosyloxy-5-phenylpentanoic Acid From *Polygonum salicifolium*

WALTER M. GANCI,[1] AYŞE KURUÜZÜM,[2] İHSAN ÇALIŞ,[2] AND PETER RÜEDI[1]*
[1]*University of Zurich, Institute of Organic Chemistry, Zürich, Switzerland*
[2]*Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey*

ABSTRACT The absolute configuration of the title compound has been determined after its enzymatic hydrolysis to 3-hydroxy-5-phenylpentanoic acid, esterification, and identification of the enantiomerically pure methyl (3*R*)-hydroxy-5-phenylpentanoate by HPLC on Chiralcel®*OD-H*. For reasons of inconsistent literature data, enantioselective reductions of methyl 3-oxo-5-phenylpentanoate have been reinspected and the stereochemical outcome unequivocally confirmed by both chiroptical and HPLC retention data. *Chirality 12:139–142, 2000.*

KEY WORDS: methyl (+)-(*R*)- and (–)-(*S*)-3-hydroxy-5-phenylpentanoates; absolute configuration; chiral HPLC; enantioselective hydrogenation; baker's yeast reduction

Phytochemical investigation of the Turkish medicinal plant *Polygonum salicifolium* (*Polygonaceae*) has led to the isolation of the genuine (–)-3-*O*-β-D-glucopyranosyloxy-5-phenylpentanoic acid (**1**, $[\alpha]_D^{23}$ = –7.5 (c 0.22, MeOH)).[1] Enzymatic hydrolysis of **1** with β-glucosidase afforded 3-hydroxy-5-phenylpentanoic acid **(2)** (Scheme 1) and HPLC analysis of the hydroxy ester **3** on Chiralcel®*OD-H* (hexane/2-propanol 12:1) gave a single peak (k' = 3.52) suggesting the enantiomeric purity of the compound. Unfortunately, a reliable direct determination of the absolute configuration by comparison of the chiroptical data of **2** and **3** was not feasible due to both the limited availability of **1** and the small specific optical rotation value expected from the literature data.[2] This fact compelled us to assign the absolute configuration of the natural product derivative by HPLC. Moreover, in the course of the literature search it turned out that there is considerable inconsistency concerning the chiroptical data and, consequently, the absolute configuration of the 3-hydroxy-5-phenylpentanoic acids and their methyl esters (**3** and *ent*-**3**).[2–4] For that reason, the stereochemical outcome of enantioselective reductions of the corresponding 3-oxo precursor **(4)** was analyzed independently in our laboratory again. In order to establish the chiroptical and HPLC retention data of these compounds with respect to the assignment of their absolute configuration, and to guarantee the consistency of the results, directly comparable experimental conditions were applied.



Scheme 1

MATERIALS AND METHODS

General

Analytical HPLC was performed on Chiralcel®*OD-H,* 5 µ (Daicel Chemical Industries, Ltd.), 250 × 4.6 mm with hexane/2-propanol (12:1), flow-rate = 0.5 ml/min, λ_{det} = 220 nm, T = 25°C. Equipment: Pharmacia LKB HPLC-Pump 2248; Hewlett-Packard HP 1040M diode array detection system; data handling on the Hewlett-Packard HP Chem Station for LC, Rev. A.04.02. Preparative HPLC was performed on Chiralcel®*OD,* 10 µ (Daicel Chemical Industries, Ltd.), 250 × 20 mm with hexane/2-propanol (15:1), flow-rate = 5 ml/min, λ_{det} = 220 nm, T = 25°C. Equipment: Applied Biosystems 400 Solvent Delivery System, Applied Biosystems 783A Programmable Absorbance Detector. The *ee* determinations are based on the integration of the peak areas of the analytical HPLC separations. The $[\alpha]_D$ values were measured at T = 25°C on a Perkin-Elmer 241-MC polarimeter with a B. Braun Thermomix *1441* thermostat in a 10-cm cell.

Isolation of (3R)-O-β-D-glucopyranosyloxy-5-phenylpentanoic acid (1)

The chromatographic purification (polyamide, SiO_2, and RP-18) of **1** from a methanolic extract of dried aerial parts

*Correspondence to: Prof. Dr. Peter Rüedi, University of Zurich, Institute of Organic Chemistry, Winterthurerstr. 190, CH-8057 Zurich, Switzerland. E-mail: peru@oci.unizh.ch
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of *Polygonum salicifolium* and the physical data are detailed in Ref. 1.

Enzymatic Hydrolysis of 1 → Methyl Ester 3

A solution of the glucoside **1** (9 mg) in acetate buffer (pH 4.4, 10 ml) was treated with β-glucosidase (20 mg) and left at 37°C for 48 h. The solution was evaporated to dryness and the residue chromatographed (SiO_2, CH_2Cl_2/MeOH/H_2O 90:10:1) to afford **2** (4 mg). The hydroxy acid **2** was methylated with CH_2N_2/Et_2O and purified (SiO_2, hexane/AcOEt 2:1) to yield **3** (3 mg). HPLC k' = 3.52, *ee* > 99%. ^{1}H-NMR and further physical data, see Ref. 1.

Methyl 3-Oxo-5-phenylpentanoate

The starting material **(4)** was prepared according to Huckin and Weiler.[5]

(±)-Methyl 3-Hydroxy-5-phenylpentanoate

A solution of **4** (130 mg, 0.6 mmol) and $NaBH_4$ (24 mg, 1.0 eq.) in MeOH was stirred at r.t. overnight. After work-up, the crude product was chromatographed (SiO_2, hexane/Et_2O 2:1) to yield 75 mg (58%) of (±)-**3** as a colorless oil. HPLC k' = 3.06 and 3.52 (R_s = 1.7, 1:1 each).

Enantioselective Hydrogenation of Methyl 3-Oxo-5-phenylpentanoate → 3 and ent-*3*

A mixture of **4** (188 mg, 0.91 mmol) and (+)-(*R*)-BINAP-Ru(II) (10 mg) in abs. EtOH (20 ml) was hydrogenated (30 bar H_2, 100°C, 24 h), then filtered over Celite and the filtrate evaporated. The residue was chromatographed (SiO_2, hexane/Et_2O 2:1) yielding 170 mg (82%) of **3** as a colorless oil. $[\alpha]_D^{25}$ = +1.40 (c 2.9, CH_2Cl_2). HPLC k' = 3.52, *ee* = 72%. Analogous reduction of **4** (155 mg, 0.75 mmol) by (–)-(*S*)-BINAP-Ru(II)/H_2 yielded 210 mg (100%) of *ent*-**3** as a colorless oil. $[\alpha]_D^{25}$ = –1.51 (c 2.9, CH_2Cl_2). HPLC k' = 3.06, *ee* = 92%.

Although our *ent*-**3** has nearly the same *ee* as the compound reported,[2] we did not obtain the significantly higher specific optical rotation value ($[\alpha]_D$ = –2.6, *ee* = 93%). In order to compare the $[\alpha]_D$ values reliably with literature data, **3** (*ee* = 72%) and *ent*-**3** (*ee* = 92%) were purified by preparative chiral HPLC followed by bulb-to-bulb distillation (80°C, 10 Pa) to yield the enantiomerically pure compounds **3** ($[\alpha]_D^{25}$ = +3.40 (c 1, CH_2Cl_2) and $[\alpha]_D^{25}$ = +3.34 (c 1, $CHCl_3$)) and *ent*-**3** ($[\alpha]_D^{25}$ = –3.40 (c 1, CH_2Cl_2) and $[\alpha]_D^{25}$ = –3.35 (c 1, $CHCl_3$)).

Biological Reduction of Methyl 3-Oxo-5-phenylpentanoate

A suspension of lyophilized commercial baker's yeast (16 g, Fleischmann's type in cubes, supermarket grade) in H_2O (50 ml) and **4** (200 mg, 0.97 mmol) was stirred for 4 days at 30°C. The mixture was then filtered over Celite, the residue washed (H_2O), the filtrate evaporated, and the residue extracted with Et_2O/2N-HCl. The organic layers were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness, affording 87 mg (46%) of **2** as a light-brown powder ($[\alpha]_D^{25}$ = +1.32 (c 1.0, CH_2Cl_2). Esterification of **2** (42 mg, 0.22 mmol) with CH_2N_2/Et_2O and purification of the crude product (SiO_2, Hexane/Et_2O 2:1), yielded 36 mg (80%) of **3** as a colorless oil. $[\alpha]_D^{25}$ = +1.58 (c 1.7, CH_2Cl_2). HPLC k' = 3.52, *ee* = 64%.

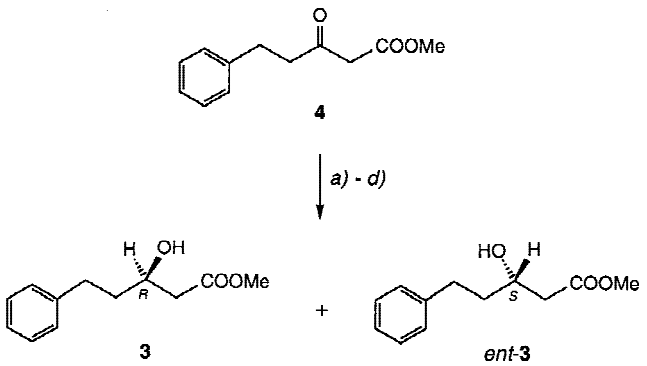
RESULTS AND DISCUSSION

In order to correlate the chromatographic and the chiroptical data with the respective absolute configuration, methyl 3-oxo-5-phenylpentanoate **(4)** was reduced by various agents to the 3-hydroxy esters **3** and *ent*-**3** (Scheme 2), and the products were analyzed by HPLC on Chiralcel®*OD-H* (hexane/2-propanol 12:1). The enantiomers (**3**/*ent*-**3**) are resolved better than baseline (R_s = 1.7), thus enabling reliable *ee* determinations and unambiguous assignments. The absolute configurations were inferred from the established stereochemical course of enantioselective reductions of such oxo esters. Hence, it is predicted that hydrogenation of **4** with (+)-(*R*)-BINAP-Ru(II)/H_2[6] and biological reduction with baker's yeast[7] would predominantly yield the (*R*)-enantiomer **(3),** whereas (–)-(*S*)-BINAP-Ru(II)/H_2 would afford mainly the (*S*)-compound (*ent*-**3**).[6] The results are summarized in Table 1.

Since the $[\alpha]_D$ values significantly differed from others reported,[2,3] we purified compounds **3** (*ee* = 72%) and *ent*-**3** (*ee* = 92%) by preparative chiral HPLC on Chiralcel®*OD* (hexane/2-propanol 15:1) to obtain the enantiopure **3** ($[\alpha]_D^{25}$ = +3.40 (c 1.0, CH_2Cl_2)) and *ent*-**3** ($[\alpha]_D^{25}$ = –3.40 (c 1.0, CH_2Cl_2)). Measuring the $[\alpha]_D^{25}$ in $CHCl_3$ changed the values only marginally (see Materials and Methods).

From the comparison of the HPLC data of the prepared **3** (k' = 3.52) and *ent*-**3** (k' = 3.06), we ambiguously conclude that the derivative of the natural product (k' = 3.52) is **3** and has (*R*)-configuration. As a consequence, the constituent of *Polygonum salicifolium* is (–)-(3*R*)-*O*-β-D-glucopyranosyloxy-5-phenylpentanoate acid **(1)**.[1]

Our findings are largely in accordance with the recent results of Spino et al.,[2] which have shown reliably that asymmetric hydrogenation of **4** with (–)-(*S*)-BINAP-Ru(II)/H_2 yielded the (–)-(*S*)-hydroxy ester (*ent*-**3,** $[\alpha]_D$ = –2.6 (c 2.71, CH_2Cl_2), *ee* = 93%), whereas biological reduction with baker's yeast afforded the (+)-(*R*)-enantiomer (**3,** $[\alpha]_D$ = +1.22 (c 0.98, CH_2Cl_2), *ee* > 80%, after methylation of **2**). The analogous reduction with (+)-(*R*)-BINAP-Ru(II)/H_2 was not performed, but the alternate procedure using (+)-(*R*)-BITIANP-Ru(II)/H_2 yielded the (+)-(*R*)-hydroxy ester **3** ($[\alpha]_D$ = +2.7 (c 2.89, CH_2Cl_2), *ee* > 98%). The absolute



Scheme 2

TABLE 1. HPLC and chiroptical properties of the reduction products of methyl 3-oxo-5-phenylpentanoate (4) under various conditions

Reducing agent	k'[a]	(*R*)/(*S*)[b]	*ee* (%)	$[\alpha]_D^{25}$	Product[a]
a) $NaBH_4$	3.06/3.52	50/50	—	—	**3**/*ent*-**3**
b) Baker's yeast	3.52	77/23	64	+1.58[c]	**3**
c) (+)-(*R*)-BINAP-Ru(II)/H_2	3.52	86/14	72	+1.40[d]	**3**
d) (–)-(*S*)-BINAP-Ru(II)/H_2	3.06	4/96	92	–1.51[d]	*ent*-**3**
Natural source	3.52		>99	—[e]	**3**
Enantiopure compounds	3.52		>99	+3.40[f]	**3**
	3.06		>99	–3.40[f]	*ent*-**3**

[a]Major enantiomer.
[b]Determined according to area %.
[c]C 1.7, CH_2Cl_2.
[d]C 2.9, CH_2Cl_2.
[e]Not determined due to the limited amount of the natural sample.
[f]After purification on Chiralcel OD,® c 1.0, CH_2Cl_2.

configurations and *ee* values have been inferred by ^{19}F-NMR spectroscopy of the respective Mosher-esters.[2]

This assignment is strongly supported by the work of Harada's group.[3] In the course of their studies on the enantioseparation of (±)-3-hydroxy-5-phenylpentanoic acid ((±)-**2**) by the (–)-menthone-derived 1,3-dioxanones, an independent determination of the absolute configuration of **3** and *ent*-**3** was carried out. Based on the ^{1}H-NMR chemical shift differences of the diastereoisomeric spiroacetals, the dextrorotatory hydrolysis product ($[\alpha]_D^{24}$ = +14.0 (c 1.04, $CHCl_3$)) was determined to have the (3*R*)-configuration and vice versa ($[\alpha]_D^{24}$ = –14.5 (c 1.02, $CHCl_3$)). Since esterification of the 3-hydroxy acids does not change the sign of the optical rotation and affects its value only marginally (see Materials and Methods), the final assignments for the corresponding esters **3** and *ent*-**3** can be made in terms as above (**3** is (+)-(*R*), *ent*-**3** is (–)-(*S*)). Their correlation of the chiroptical data with the absolute configuration is the same as ours, but the values of the specific optical rotation reported are significantly higher than those of our compounds, which are enantiopure by chiral HPLC.

In addition, conflicting results have been reported by Fujisawa et al.[4] The reduction of chiral sulfoxides with diisobutylaluminium hydride (DIBAH) under different reaction conditions is the key source of a contradictory assignment of the absolute configuration of the optically active methyl 3-hydroxy-5-phenylpentanoates. Reduction of (*S*)-1-(*p*-chlorophenylsulfinyl)-4-phenyl-2-butanone with DIBAH is stated to afford (2*R*)-1-[(*S*)-1-(*p*-chlorophenylsulfinyl)]-4-phenyl-2-butanol, whereas reduction with DIBAH/$ZnCl_2$ yields the (2*S*)-diastereoisomer. As a consequence, further transformations of these compounds (carboxylation, elimination of the sulfinyl auxiliary, esterification) are reported to give methyl (3*R*)-hydroxy-5-phenylpentanoate which is levorotatory ($[\alpha]_D^{23}$ = –12.2 (c 1.16, $CHCl_3$)) and the dextrorotatory (3*S*)-enantiomer ($[\alpha]_D^{23}$ = +12.2 (c 0.98, $CHCl_3$)). However, based on the results mentioned above, this divergent assignment should be ruled out and either the proposed mechanistic pathway or the absolute configuration of the starting material is questionable.

In connection with this discussion of contradictory chiroptical data, it is worthy of note that enantioselective reduction of the related ethyl 2-oxo-4-phenylbutanoate with either (+)-(*R*)-BINAP-Ru(II)/H_2 or baker's yeast was shown[8] to yield the expected[6,7] (2*R*)-enantiomer ($[\alpha]_D^{20}$ = –14.5 (c 2.6, $CHCl_3$, *ee* = 97%),[8a] $[\alpha]_D^{25}$ = –15.1 (c 1.0, $CDCl_3$, *ee* > 99%)[8b]). It is noteworthy that both the sign and magnitude of the specific optical rotation changes from the (3*R*)-hydroxy-5-phenylpentanoic to the (2*R*)-hydroxy-4-phenylbutanoic acid derivatives, although both compounds have the same absolute stereochemistry.

Recently, (–)-3-*O*-β-D-glucopyranosyloxy-5-phenylpentanoic acid has been reported to be a constituent of *Perilla frutescens,* but the absolute configuration remained undetermined.[9] Although the specific optical rotation ($[\alpha]_D^{20}$ = –10.5 (c 0.06, MeOH)) suggests it to be the (3*R*)-enantiomer, the (3*S*)-diastereoisomer cannot be precluded as the influence of the glucose moiety on the chiroptical data is not known a priori.

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