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Synthesis of acetyldimethyl(phenyl)silane and its enantioselective conversion into (*R*)-(1-hydroxyethyl)dimethyl(phenyl)silane by plant cell suspension cultures of *Symphytum officinale* L. and *Ruta graveolens* L.

Reinhold Tacke, Stephan A. Wagner, Susanne Brakmann and Frank Wuttke

Institut für Anorganische Chemie, Universität Karlsruhe, Engesserstraße, Geb. 30.45, D-76128 Karlsruhe (Germany)

Udo Eilert

Institut für Pharmazeutische Biologie, Technische Universität Braunschweig, Mendelssohnstraße 1, D-38106 Braunschweig (Germany)

Lutz Fischer and Christoph Syldatk

Institut für Biochemie und Biotechnologie, Technische Universität Braunschweig, Spielmannstraße 7, D-38106 Braunschweig (Germany)

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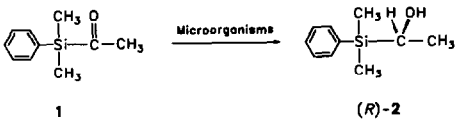
Abstract

Starting from chlorodimethyl(phenyl)silane (**3**), acetyldimethyl(phenyl)silane (**1**) was prepared by a two-step synthesis in a total yield of 90% [$PhMe_2SiCl$ (**3**) → $PhMe_2SiC(OMe)=CH_2$ (**4**) → $PhMe_2SiC(O)Me$ (**1**)]. The prochiral acetylsilane **1** was transformed enantioselectively into (*R*)-(1-hydroxyethyl)dimethyl(phenyl)silane [(*R*)-**2**] using plant cell suspension cultures of *Symphytum officinale* L. or *Ruta graveolens* L. Under preparative conditions (300-mg scale, not optimized), (*R*)-**2** was isolated in 15% (*Symphytum*) and 9% yield (*Ruta*), respectively. The enantiomeric purities of the products were 81% ee (*Symphytum*) and 60% ee (*Ruta*), respectively.

1. Introduction

In 1983 we demonstrated for the first time that biotransformations can be used as a synthetic method in organosilicon chemistry [1]. Subsequently, many papers dealing with microbial transformations or enzymatic conversions of organosilicon compounds have appeared (for our investigations see refs. 2–17 and for studies by other groups see refs. 18–26). In some of these investigations, it was shown that biotransformations can be used for the synthesis of optically active silanes with the silicon atom as the centre of chirality [2–4,6,8,15,16,22].

In the course of our systematic studies on biotransformations of organosilicon compounds, we have demonstrated that acetyldimethyl(phenyl)silane (**1**) can be transformed enantioselectively into (*R*)-(1-hydroxyethyl)dimethyl(phenyl)silane [(*R*)-**2**] using various strains of microorganisms (bacteria, fungi, yeasts, cyanobacteria, green algae) (Scheme 1) [5,7,11,12]. Here we report the first results arising from use of plant cell suspension cultures to bring about this reaction. We describe (i) a new synthesis of the prochiral acetylsilane **1** (for the first synthesis, see [27]) and (ii) its enantioselective conversion into the corresponding (1-hydroxyethyl)silane (*R*)-**2** by plant cell suspension cul-



1 (*R*)-**2**

Scheme 1

Correspondence to: Prof. Dr. R. Tacke.

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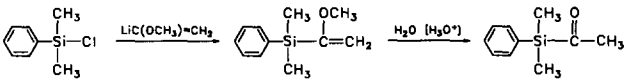
tures of *Symphytum officinale* L. or *Ruta graveolens* L. This is, to the best of our knowledge, the first report of biotransformations of an organosilicon substrate involving suspension cultures of whole plant cells.

2. Results and discussion

Starting from chlorodimethyl(phenyl)silane (**3**), acetyldimethyl(phenyl)silane (**1**) was prepared by a two-step synthesis as shown in Scheme 2 [28]. In the first step, (1-methoxyvinyl)dimethyl(phenyl)silane (**4**) was synthesized in 92% yield by reaction of **3** with (1-methoxyvinyl)lithium. In the second step, the (1-methoxyvinyl)silane **4** was transformed in 98% yield into the corresponding acetylsilane **1** by an acid-catalyzed hydrolysis. This synthesis of **1** (total yield 90%) is more advantageous than that described in [27] (better yield; avoidance of mercury and cadmium salts).



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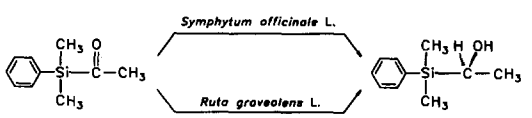
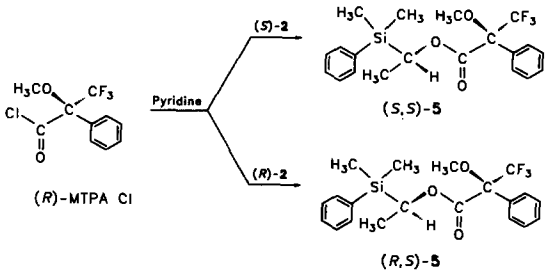
Scheme 2

The prochiral acetylsilane **1** was reduced enantioselectively with plant cell suspension cultures of *Symphytum officinale* L. or *Ruta graveolens* L., to give the corresponding (*R*)-(1-hydroxyethyl)silane (*R*)-**2** (Scheme 3). The biotransformations were carried out at 25°C on a preparative scale (300-mg scale) in an acidic medium [pH 5.6 (*Symphytum*); pH 5.1 (*Ruta*)] in the dark. In both cases the substrate concentration was 0.6 g/l. After an incubation time of 4 h, no more substrate was detectable (GLC control). The cell mass was subsequently separated by centrifugation, and the biotransformation products were isolated from the aqueous phase by extraction with ethyl acetate and were then purified by column chromatography on silica gel [yield 15% (*Symphytum*) and 9% (*Ruta*), respectively; enantiomeric purity 81% ee (*Symphytum*) and 60% ee (*Ruta*), respectively]. Considerable amounts of dimethyl(phenyl)silanol ($PhMe_2SiOH$) and tetramethyl-1,3-diphenyldisiloxane ($PhMe_2SiOSiMe_2Ph$), respectively, were detected as by-products of these bioconversions. It is not clear whether these compounds were formed chemically or enzymatically.



1 (*R*)-2

Scheme 3



Scheme 4

The absolute configuration and enantiomeric purity of the biotransformation products were determined, after derivatization with (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA Cl] (Scheme 4), by ^1H and ^{19}F NMR spectroscopic studies of the corresponding MTPA esters using the general method described in [29]. For purposes of comparison, racemic **2** (*rac*-**2**; available according to [5]) and a 1:1 mixture of the corresponding diastereomeric MTPA esters (*R*,*S*)-**5** and (*S*,*S*)-**5** [available by esterification of *rac*-**2** with (*R*)-MTPA Cl [30]; Scheme 4] were prepared.

The enantiomeric purities of the biotransformation products (81% ee and 60% ee, respectively) were similar to those observed in most of the previously reported microbial reductions (see [5,7,11,12]). No further attempts to improve the poor yields of (*R*)-**2** (15% and 9%, respectively) were made. The aim of this study was to demonstrate that plant cell suspension cultures can be used for the synthesis of optically active organosilicon compounds.

3. Experimental

3.1. Chemical syntheses

All syntheses were performed under dry nitrogen using dried organic solvents. The ^1H, ^{13}C and ^{29}Si NMR spectra of **1**, **2** and **4** were recorded on a Bruker WM-400 (^1H, 400.1 MHz; ^{13}C, 100.6 MHz) or a Bruker AC-200 spectrometer (^{29}Si, 39.7 MHz). Chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^1H, δ 7.25), $CDCl_3$ (^{13}C, δ 77.05) and TMS (^{29}Si, δ 0). Assignment of the ^{13}C data was supported by DEPT experiments; the results of these experiments are included in the assignments. The ^1H and ^{19}F NMR spectra of **5** were recorded on a Bruker AC-250 spectrometer at 250.1 and 235.3 MHz, respectively; chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^1H, δ 7.25) and external $CFCl_3$ (^{19}F, δ 0). Mass spectra were obtained with a Finnigan-MAT-8430 mass spectrometer (EI MS, 70 eV); the *m/z* values given refer to the isotopes ^1H, ^{12}C, ^{16}O and ^{28}Si.

Acetyldimethyl(phenyl)silane (1)

Compound **4** (10.0 g, 52.0 mmol) was added dropwise during 2 min at 20°C to a stirred mixture of acetone and 1 M hydrochloric acid (30 ml; 4:1, v/v). After 70 min stirring at 20°C (exclusion of light), diethyl ether (50 ml) and water (50 ml) were added and the mixture was shaken. The organic layer was subsequently separated and the aqueous phase extracted twice with diethyl ether (2 × 25 ml). The combined organic extracts were washed three times with water (3 × 10 ml) and then dried over Na_2SO_4. The solvent was removed under reduced pressure (rotary evaporator) and the residue subsequently purified by Kugelrohr distillation (oven temperature 52°C, 0.4 Torr) to yield 9.1 g (98%) of a pure (^1H and ^{13}C NMR) liquid product, which was stored at −20°C (exclusion of light). ^1H NMR ($CDCl_3$): δ 0.48 (s, 6H, $SiCH_3$); 2.21 (s, 3H, CCH_3); 7.3–7.4 and 7.5–7.6 (m, 5H, SiC_6H_5). ^{13}C NMR ($CDCl_3$): δ −4.9 ($SiCH_3$); 35.9 (CCH_3); 128.2 (C_m, SiC_6H_5); 129.9 (C_p, SiC_6H_5); 133.9 (C_o, SiC_6H_5); 134.3 (C_i, SiC_6H_5); 207.9 (CO). ^{29}Si NMR ($CDCl_3$): δ −16.5. MS: *m/z* 178 (6%, M^+), 135 (100%, $M^+ - C(O)CH_3$). $C_{10}H_{14}OSi$ (178.3).

rac-(1-Hydroxyethyl)dimethyl(phenyl)silane (rac-2)

This was prepared as described in [5].

Chlorodimethyl(phenyl)silane (3)

This was a gift from the Bayer AG (Leverkusen).

(1-Methoxyvinyl)dimethyl(phenyl)silane (4)

A 1.6 M solution of t-butyllithium in n-pentane (338 ml, 0.54 mol ^tBuLi) was added dropwise during 2.5 h at −78°C to a stirred solution of methyl vinyl ether (39.0 g, 0.67 mol) in tetrahydrofuran (240 ml); a yellow precipitate was formed. The mixture was allowed to warm during 3 h to 0°C [giving a colourless solution of (1-methoxyvinyl)lithium] and then cooled to −70°C. A solution of **3** (70.0 g, 0.41 mol) in tetrahydrofuran (100 ml) was added dropwise during 1.5 h at −70°C to the stirred solution of (1-methoxyvinyl)lithium, and the mixture then allowed to warm during 3 h to 20°C and stirred for 20 h at this temperature. The resulting mixture was shaken twice with a saturated aqueous solution of ammonium chloride (2 × 150 ml), the organic layer was separated, the combined aqueous phases were extracted twice with n-pentane (2 × 50 ml), and the combined organic layers were then dried over K_2CO_3. The solvent was removed under reduced pressure (rotary evaporator) and the residue purified by fractional distillation *in vacuo* (Vigreux column) to yield 72.5 g (92%) of a pure (^1H and ^{13}C NMR) liquid product; b.p. 42°C/0.05 Torr. ^1H NMR ($CDCl_3$): δ 0.39 (s, 6H, $SiCH_3$); 3.47 (s, 3H, OCH_3); 4.35 (d, $^2J(HH) = 2.1$ Hz, 1H) and 4.68 (d, $^2J(HH) = 2.1$ Hz, 1H); ($SiC{=}CH_2$); 7.3–7.4 and 7.5–7.6 (m, 5H, SiC_6H_5). ^{13}C NMR ($CDCl_3$): δ −3.6 ($SiCH_3$); 54.2 (OCH_3); 95.3 ($SiC{=}CH_2$); 127.8 (C_m, SiC_6H_5); 129.2 (C_p, SiC_6H_5); 134.0 (C_o, SiC_6H_5); 137.1 (C_i, SiC_6H_5); 168.6 ($SiC{=}CH_2$). ^{29}Si NMR ($CDCl_3$): δ −11.4. MS: *m/z* 192 (6%, M^+), 135 (100%, $M^+ - C(OCH_3){=}CH_2$). Anal. Found: C, 68.4; H, 8.6. $C_{11}H_{16}OSi$ (192.3) calc.: C, 68.69; H, 8.38%.

Transformation of rac-2 into a 1:1 mixture of the MTPA esters (R,S)-5 and (S,S)-5

(*R*)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA Cl; prepared from (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Fluka, 65364) as described in [29]] (25 μl) was added at room temperature to a stirred mixture of tetrachloromethane/pyridine (1:1, v/v; 600 μl) and *rac*-**2** (25 μl). After 24 h stirring at room temperature [complete conversion as monitored by TLC; silica gel plates (Merck, 5735), n-hexane/diethyl ether (1.8:1, v/v), UV detection], 3-dimethylamino-1-propylamine (40 μl) was added, and the mixture was stirred for 10 min. After addition of diethyl ether (10 ml) and 2 M hydrochloric acid (10 ml), the organic layer was separated and shaken first with saturated aqueous Na_2CO_3 solution (10 ml) and then with water (10 ml). The organic layer was dried carefully over $MgSO_4$, the solvent removed under reduced pressure, and the residue dissolved in $CDCl_3$ (0.5 ml). To remove traces of diethyl ether, the solvent was again evaporated off and the residue redissolved in $CDCl_3$. The sample obtained by this procedure was used for the NMR spectroscopic studies. Assignment of the absolute configuration at the asymmetrically substituted carbon atoms of the $SiC^*H(OR)CH_3$ moieties of the stereoisomers of **2** and **5** was performed by the correlation method described in [29].

(*R,S*)-**5**: ^1H NMR ($CDCl_3$): δ 0.32 (s, 3H, $SiCH_3$); 0.34 (s, 3H, $SiCH_3$); 1.30 (d, $^3J(HH) = 7.4$ Hz, 3H, CCH_3); 3.41 (q, uncomplete resolution, $^5J(HF) = 1.0$ Hz, 3H, OCH_3); 5.16 (q, $^3J(HH) = 7.4$ Hz, 1H, SiCH(OR)C); 7.3–7.6 (m, 10H, SiC_6H_5, CC_6H_5). ^{19}F NMR ($CDCl_3$): δ −71.8.

(*S,S*)-**5**: ^1H NMR ($CDCl_3$): δ 0.26 (s, 3H, $SiCH_3$); 0.29 (s, 3H, $SiCH_3$); 1.25 (d, $^3J(HH) = 7.4$ Hz, 3H, CCH_3); 3.47 (q, uncomplete resolution, $^5J(HF) = 1.0$ Hz, OCH_3); 5.18 (q, $^3J(HH) = 7.4$ Hz, 1H, SiCH(OR)C); 7.3–7.6 (m, 10H, SiC_6H_5, CC_6H_5). ^{19}F NMR ($CDCl_3$): δ −71.7.

3.2. Biotransformations

Plant cell suspension cultures

Symphytum officinale L.: The suspension culture of *Symphytum officinale* L. was established from a light

grown, friable and whitish callus. The callus was obtained in 1983 from surface sterilized stem explants on a B5-medium [31] containing 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2% sucrose. The cell culture was grown in 250-ml Erlenmeyer flasks containing 75 ml of the liquid 1 B5-medium and subcultured every 3 weeks (25°C, photoperiod 16 h/day). During the cultivation, the flasks were kept on a gyratory shaker (Infors, München, Germany) at 120 rev./min. The cells were whitish to grey, remained single or formed small aggregates and showed no signs of differentiation.

Ruta graveolens L.: The suspension culture of *Ruta graveolens* L. (R-MS) was established from a light grown, green callus in 1981, which was obtained from Professor Czygan (Würzburg, Germany) in 1977. This callus was initiated from hypocotyl explants of sterile grown seedlings. The nutrient medium used was an MS-medium [32] containing 0.1 mg/l 2,4-D, 0.25 mg/l kinetin and 3% sucrose. The cell culture was grown in 250-ml Erlenmeyer flasks containing 75 ml of the MS-medium and subcultured every 4 weeks (25°C, photoperiod 16 h/day). During the cultivation, the flasks were kept on a gyratory shaker (Infors, München, Germany) at 120 rev./min. The suspension was heterogeneous in aggregate size and tended to form large, compact clumps which were light- to dark-green. The culture showed other signs of differentiation like formation of tracheid structures and idioblasts.

Preparation of (R)-2

Suspension cultures of *Symphytum officinale* L. and *Ruta graveolens* L. were grown for 2 weeks as described above, and 500 ml of each culture were transfered separately into 1-l shaking flasks under sterile conditions. After addition of **1** (300 mg, 1.68 mmol) to each of the two flasks (substrate concentration 0.6 g/l), the cultures were incubated aerobically for 4 h at 25°C and 100 rev./min (rotary shaker RS 206, Infors AG, Switzerland). The cells (no more substrate was detectable as monitored by GLC; see below) were then harvested by centrifugation for 30 min at 6000 rev./min and 10°C (Cryofuge M, Heraeus-Christ, Osterode, Germany), the supernatant liquids extracted twice with ethyl acetate (2 × 1 l), and the solvent removed *in vacuo* (rotary evaporator). The crude products were purified by column chromatography on silica gel [column diameter 2.5 cm; 50 g silica gel 60, 0.063–0.200 mm, Merck, 7734; elution with diethyl ether/hexane (1 : 1.8, v/v)], to yield 46 mg (15%, *Symphytum*) and 28 mg (9%, *Ruta*), respectively, of a colourless liquid [enantiomeric purity 81% ee (*Symphytum*) and 60% ee (*Ruta*), respectively; the absolute configuration and enantiomeric purity of the respective biotransformation products (*R*)-**2** were determined by NMR spectroscopy after derivatization with (*R*)-MTPA Cl, as described above for *rac*-**2** [conversion into (*R*,*S*)-**5**/(*S*,*S*)-**5**)]. The ^{1}H and ^{13}C NMR spectra and the mass spectra of the purified products were identical with those of chemically prepared *rac*-**2** (see [5]).

Monitoring of the time course of the biotransformations: analytical procedure

The progress of the bioconversions was monitored by GLC as follows: after starting the biotransformations, samples of 1 ml of the culture suspensions were taken after various times of incubation and subsequently extracted with 1 ml of dichloromethane. Quantitative determination of substrate and products was performed by GLC analysis of 1 μl of each of these extracts using a Carlo Erba gas chromatograph [model GC 6000; packed OV-17 column (500 mm); temperature programme 80°C (2 min)–180°C (2 min) with 20°C/min; carrier gas nitrogen].

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