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The structure of laricinolic acid and its biomimetic transformation into officinalic acid

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Laricinolic acid (**8**), a new sesquiterpene of the drimane type, has been isolated from the wood-rotting fungus *Laricifomes officinalis*. Its structure was elucidated by spectroscopic means and confirmed *via* a correlation with the known drimenine derivative **13**. Oxidation of **8** to **1**, followed by a mild thermal treatment, furnished (−)-officinalic acid (**4**) in 65% yield. This transformation establishes the hitherto unknown absolute configuration of the latter. An independent correlation was achieved by pyrolysis of **4** which furnished (−)-dihydrooxoisodrimenine (**14**) of known absolute configuration.

Introduction

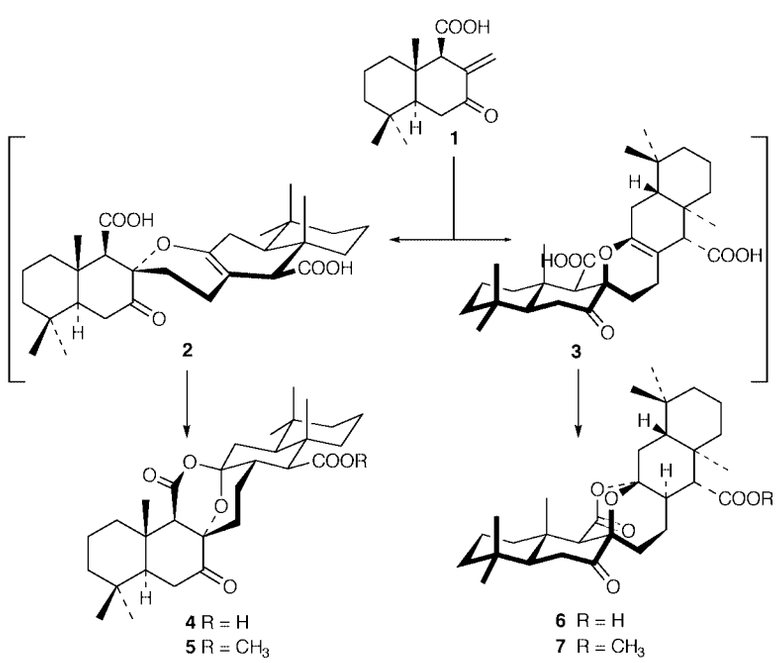
Some time ago, we reported a biomimetic synthesis of the racemic form of officinalic acid (**4**) (Scheme 1),[1] a metabolite first isolated in 1893 by Jahns[2] from the fungus *Laricifomes officinalis*. Its structure was established many years later by Epstein *et al.*[3] through a single crystal X-ray analysis of a tetrahydro derivative. In the key step of our synthesis a hetero-Diels–Alder reaction of the racemic forms of the enone acid **1** yielded a 5:1-mixture of the homodimeric enol ether **2** and its heterodimeric counterpart **3**, which underwent *in situ* lactonisation to officinalic acid (**4**) and its diastereoisomer **6**, respectively.

Results and discussion

For an unambiguous identification of our racemic synthetic officinalic acid, a reference sample of enantiomerically pure **4** was isolated from a commercially available extract of *Laricifomes officinalis*. During an examination of mother liquors from the crystallisation of **4** we noted the presence of a crystalline, optically active acid with the elemental composition $C_{15}H_{24}O_3$, mp 213 °C, $[\alpha]_D = -30.8$, for which we propose the trivial name laricinolic acid. Comparison with the data on record for the so-called "Substanz 12" (mp 215–218 °C, $[\alpha]_D = -28.6$), previously isolated from the same source by Schulte *et al.*,[4a] suggests that the two compounds may be identical, in spite of some minor deviations in their IR and ^{1}H-NMR spectra. Unfortunately, a reference sample of "Substanz 12" is no longer available for direct comparison.[4b]

According to its IR and ^{1}H-NMR spectra, laricinolic acid contains a carboxy group and a secondary hydroxy group, as well as three quaternary methyl groups and an *exo*-methylene unit. The off-resonance decoupled ^{13}C-NMR spectrum confirms the presence of these groups and, in addition, displays signals for three methine units, four saturated methylene groups and two quaternary carbon atoms.

The working hypothesis that the natural product represents a new drimane sesquiterpene was substantiated through analysis of the ^{1}H-NMR spectrum of the corresponding methyl ester **9**,



Scheme 1

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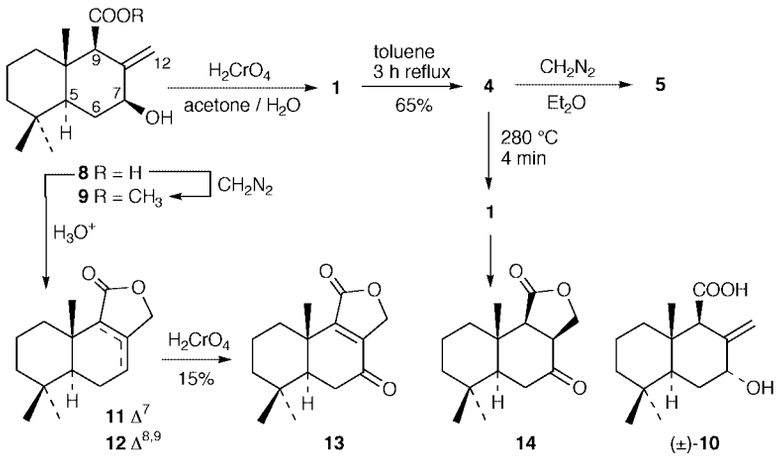
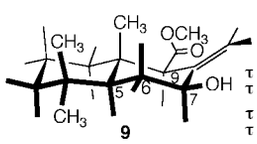


Scheme 2



	τ (5/6$_{ax}$) = 173°	τ (5/6$_{eq}$) = 70°	τ (7/6$_{ax}$) = 175°	τ (7/6$_{eq}$) = 58°

No.	d [ppm]	*J* [Hz] with: 6$_{ax}$	6$_{eq}$	7
5	1.13	12.8	2.5	<0.5
6$_{ax}$	1.35	–	12.2	10.8
6$_{eq}$	2.09	12.2	–	5.7
7	4.06	10.8	5.7	–

9

Fig. 1 Torsional angles as calculated by PM3 (MOPAC) and observed ^{1}H-NMR coupling constants of **9**. Unlabelled substituents represent hydrogen atoms.

which was corroborated by extensive decoupling experiments (see Fig. 1). Specifically, the axially oriented C(7)-hydrogen of **9** is responsible for the appearance of a doublet of doublets, centred at δ 4.06 ppm, whereas in the racemic compound (±)-**10** (Scheme 2) with an axial arrangement of the hydroxy group the signal of the equatorial C(7)-hydrogen was reported as a broad singlet at δ 4.40 ppm.[5]

The problems of the relative and absolute configuration at the C(5) and C(10) centres of laricinolic acid could be solved *via* a two-step correlation with the known (+)-7-oxodrimenine (**13**).[6] This transformation involved an acid-catalysed dehydration of **8** to give a mixture of drimenine (**11**) and isodrimenine (**12**)† which was oxidised with chromic acid to give **13**. As the sign of the specific rotation of our sample was the same as that reported by the Overton group, the absolute configuration of laricinolic acid (**8**) must be as shown in Scheme 2. During the transformation of **8** to **13** the original stereogenic centre at C(9) of **8** is destroyed, but the correlations reported below provide convincing evidence for the equatorial arrangement of the carboxy group of **8**.

The availability of **8** paved the way for a configurational correlation with natural officinalic acid (**4**) through a biomimetic synthesis of the latter. To this end, compound **8** was oxidised to the crystalline but rather unstable enone **1**, which was subjected without purification to mild thermal treatment (3 h reflux in toluene). Chromatography of the resulting crude product furnished a pure compound, identical in every respect with the naturally occurring (−)-officinalic acid (**4**), thus establishing the hitherto unknown absolute configuration of this compound. The yield obtained in the conversion of **1** into **4** (65% over both steps) is considerably higher than the one previously observed for the dimerisation of racemic **1** (46%); in addition, no trace of a compound corresponding to isoofficinalic acid (**6**) could be detected in this experiment, a finding that can be taken as an indirect, but nevertheless compelling argument in support of the claim that this stereoisomer of officinalic acid (**4**) is assembled from two enone units (**1**) of opposite absolute configuration.

A second configurational correlation was later discovered by the observation that officinalic acid (**4**), when heated above its melting point, decomposes to a mixture containing **1** and the known (−)-dihydrooxodrimenine (**14**).[6] This transformation, which provides independent proof for the absolute configuration of **4**, must involve an acid-catalysed opening of the lactone ring, followed by a retro-Diels–Alder reaction of the resulting enol ether **2** to provide **1** which then undergoes an unusual[7] 5-*endo*-*trig*-lactonisation to **14**.

Conclusion

The co-occurence of laricinolic acid (**8**) and officinalic acid (**4**) in *Laricifomes officinalis* and the demonstration that **1**, the oxidised form of **8**, undergoes specific dimerisation to **4** lends credence to the hypothesis that this transformation is an excellent mimic for the formation of **4** in the fungus. The specificity and relative ease of this dimerisation raises the interesting question of whether or not this process requires participation of a specific "Diels–Alderase" in the fungus.

Experimental

Melting points were determined in sealed evacuated capillaries. Specific rotations were measured in a 10 cm quartz tube on a Perkin-Elmer polarimeter, model 141. $[\alpha]_D$ has units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin-Elmer, model 125, as 3–5% solutions in chloroform, unless stated otherwise. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker WM 300 spectrometer (300 MHz), unless stated otherwise. ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker WM 300 spectrometer (75 MHz), unless stated otherwise. *J* values are given in Hz. Mass spectra were recorded on a Hitachi Perkin-Elmer spectrometer (model RMU-6A) using EI (70 eV); relative intensities are given in % of the base peak.

Isolation of laricinolic acid (8)

Continuous Soxhlet extraction of 2.63 kg of pulverised *Laricifomes officinalis* (Siegfried AG, Zofingen, Switzerland) with low-boiling petroleum ether for 10 days furnished 106.2 g of a crude extract which was dissolved in 200 ml of petroleum ether and kept at 4 °C for 2 weeks. The resulting precipitate (23.9 g) was suspended in 200 ml of a warm toluene–ethyl acetate mixture (10 : 1). The insoluble part (1.21 g) was discarded and the residue of the evaporated solution was purified by chromatography using toluene–ethyl acetate (20 : 1 to 1 : 1) as eluent. The fractions with r_F = 0.26–0.52, toluene–ethyl acetate (1 : 1), were pooled, resulting in 2.54 g of an amorphous material. Crystal-

† In keeping with the equatorial orientation of the hydroxy group, compound **8** proved more resistant to dehydration than its epimeric racemic counterpart **10**.

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lisation from benzene–acetone gave 186 mg of crude **4** which was recrystallised from CH_2Cl_2–acetone to give 78 mg of pure **4**. The combined mother liquors were dissolved in ether and extracted with aq. $NaHCO_3$ to give 142 mg of an acidic fraction which after crystallisation from hexane–acetone and recrystallisation from $CHCl_3$–acetone delivered 78 mg of pure **8**, $[\alpha]_D = -30.8$ (*c*, 1.12, EtOH); mp 213 °C (Found: C, 71.23; H, 9.39. $C_{15}H_{24}O_3$ requires C, 71.39; H 9.59%); ν_{max} (KBr)/cm^{-1} 3600–2500, 2950, 2920, 2860, 1714, 1694, 1448, 1460, 1368, 1308, 1211, 1183, 1088, 905, 830, 688, 653; δ_H (d_6-acetone) 0.86 (3H, s, *Me*), 0.92 (3H, s, *Me*), 1.05 (3H, s, *Me*), 1.16–1.29 (3H, m, including 1.22 (1H, dd, *J* 12.9, 2.2), 1.35 (1H, ddd, *J* 12.9, 12.0, 10.8), 1.40–1.53 (6H, m), 2.04 (1H, ddd, *J* 12.0, 5.6, 2.2), 2.73 (1H, br s), 4.04 (1H, br dd, *J* 10.8, 5.6), 4.90 (1H, m), 5.26 (1H, m); δ_C (d_6-acetone) 14.5 (q), 19.6 (t), 22.0 (q), 33.1 (t), 33.7 (q), 33.8 (s), 39.3 (t), 39.6 (s), 42.5 (t), 53.0 (d), 61.4 (d), 72.8 (d), 105.5 (t), 147.7 (s), 172.5 (s); *m*/*z* (EI) 252 (7%), 234 (41), 219 (25), 191 (16), 189 (10), 173 (12), 137 (56), 123 (100), 119 (24), 109 (48), 105 (23), 95 (39), 81 (45), 69 (79).

Methyl laricinolate (9)

A solution of 26.2 mg (0.1 mmol) of **8** in 5 ml of CH_2Cl_2 was treated with a slight excess of a freshly distilled ethereal solution of diazomethane. The solvents were removed and the residue distilled (95 °C/0.01 Torr) to yield 26 mg (0.098 mmol, 98%) of a colourless viscous oil, $[\alpha]_D = -19.7$ (*c*, 1.37, EtOH) (Found: C, 71.89; H, 9.70. $C_{16}H_{26}O_3$ requires C, 72.14; H 9.84%); ν_{max} ($CHCl_3$)/cm^{-1} 3610, 2955, 2850, 1735, 1650, 1435, 1392, 1371, 1265, 1164, 1019, 908; δ_H 0.84 (3H, s, *Me*), 0.90 (3H, s, *Me*), 1.05 (3H, s, *Me*), 1.10–1.28 (3H, m, including 1.13 (1H, dd, *J* 12.8, 2.5)), 1.35 (1H, ddd, *J* 12.8, 12.2, 10.8), 1.42–1.70 (4H, m), 1.90 (1H, br s O*H*), 2.09 (1H, ddd, *J* 12.2, 5.7, 2.5), 2.72 (1H, br s), 3.66 (3H, s O*Me*), 4.06 (1H, br dd, *J* 10.8, 5.7), 4.90 (1H, m), 5.19 (1H, m); δ_C 14.2 (q), 18.9 (t), 21.7 (q), 33.1 (t), 33.3 (s), 33.4 (q), 38.6 (t), 39.3 (s), 41.8 (t), 51.1 (q), 52.6 (d), 61.1 (d), 72.8 (d), 105.4 (t), 145.8 (s), 171.4 (s); *m*/*z* (EI) 266 (12%), 248 (29), 233 (17), 205 (10), 189 (15), 173 (19), 137 (43), 129 (74), 123 (100), 119 (29), 109 (36), 97 (58), 81 (50), 69 (84).

Transformation of laricinolic acid (8) into 6-oxoisodrimenine (13)

To a solution of 38 mg (0.15 mmol) of **8** in 5 ml of 1,4-dioxane were added 0.7 ml of 6 M H_2SO_4. After refluxing for 24 h the cooled solution was treated with 30 ml of saturated aq. $NaHCO_3$ solution and the mixture extracted 3 times with ether. The combined extracts were dried ($MgSO_4$), filtered and evaporated to yield 22 mg of a neutral fraction, containing a mixture of **11** and **12** (TLC-evidence). This mixture was dissolved in 1.2 ml of AcOH and treated with 0.2 ml of Beckmann's mixture (10 g $K_2Cr_2O_7$, 8.7 g conc. H_2SO_4 and 50 ml H_2O). After stirring at 25 °C for 16 h the mixture was poured onto crushed ice and extracted with ether. Preparative thin layer chromatography of the resulting crude mixture using hexane–ethyl acetate (5:1) as eluent furnished 4.5 mg (0.018 mmol, 12%) of **13**. Crystallisation from ether–hexane gave 3.6 mg of colourless prisms, $[\alpha]_D = +41.6$ (*c*, 0.14, benzene) (lit.[6] +52 (*c*, 1.89, benzene)); mp 112 °C (lit.[6] 112–113 °C); ν_{max}, δ_H, λ_{max}, and *m*/*z* values were identical with those obtained previously for racemic **13**.[1]

Transformation of laricinolic acid (8) into officinalic acid (4)

To a solution of 23 mg (0.091 mmol) of **8** in 3.5 ml of acetone were added 0.1 ml (5.9 equiv.) of Jones' reagent at 0 °C. After stirring for 5 min, excess reagent was destroyed by addition of a few drops of EtOH. The mixture was poured onto crushed ice and extracted 3 times with ether. The combined extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated to yield 24 mg of a crystalline residue (mp 233–235 °C, decomp.), consisting of essentially pure **1** which showed UV, IR and ^{1}H-NMR data identical with those observed for racemic **1**.[1] This material was dissolved in 3 ml of toluene and heated for 3 h at reflux. The residue was recrystallised from CH_2Cl_2–acetone to yield 15 mg (0.03 mmol, 65%) of **4** in the form of long needles, mp 268 °C (mixed mp with natural **4** 267 °C). The mother liquors were treated with diazomethane and analysed by TLC, toluene–ethyl acetate (20:1). The major compound was shown to be **5** ($r_F = 0.28$), and no methyl isoofficinalate (**7**) ($r_F = 0.21$) could be detected.

Thermolysis of officinalic acid (4)

Crystalline **4** (17 mg, 0.034 mmol) was heated for 5 min in a small glass tube under argon in a sublimation block, preheated to 275–280 °C. TLC-analysis of the crude material indicated the presence of some starting material, and of the known compounds **1** and **14**. Chromatography of the cooled mixture using hexane–ethyl acetate (3:1) as eluent gave 6 mg (0.024 mmol, 35%) of **14** which was recrystallised from hexane to yield 4.2 mg of colourless needles, $[\alpha]_D = -98.6$ (*c*, 0.43, benzene) (lit.[6] −115 (*c*, 1.0, benzene)); mp 124 °C (lit.[6] 124–126 °C); ν_{max}, δ_H, and *m*/*z* values were identical with those obtained previously for racemic **14**.[1]

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