3',4-Di-*O*-methylcedrusin: synthesis, resolution and absolute configuration

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The title compound has been synthesised in racemic form by a biomimetic reaction sequence. The two enantiomers were resolved by column chromatography of one of the synthetic intermediates. On the basis of CD results a tentative absolute configuration for the synthetic enantiomers and natural 3',4-di-*O*-methylcedrusin is proposed.

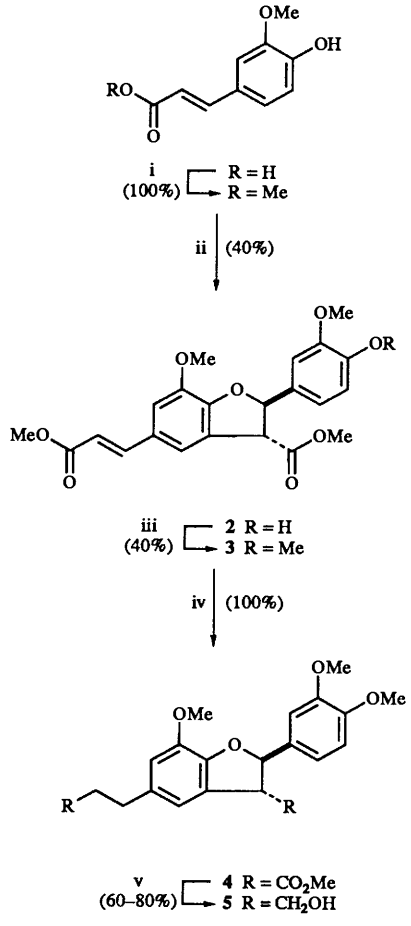
Sangre de drago (dragon's blood), a blood-red latex produced by various South American *Croton* species, is widely used in local medicine for its wound-healing properties and as an anti-cancer agent. 3',4-Di-*O*-methylcedrusin or 3-[2-(3,4-dimethoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol **5** has been shown to be one of the active principles being a wound healing agent and an inhibitor of thymidine incorporation in endothelial cells.[1,2]

Results and discussion

Racemic **5**[3] has been synthesised as shown in Scheme 1. Near quantitative esterification of ferulic acid was achieved with a heterogeneous polymer catalyst[5] to give methyl ferulate[4] **1** the biomimetic[6,7] oxidative coupling of which, in the presence of silver oxide,[8] to generate the dihydrobenzofuran skeleton is the crucial step in the reaction sequence. Compound **2**, shown unequivocally by X-ray crystallography to have a *trans*-configuration,[9] upon attempted methylation with diazomethane or with dimethyl sulfate gave complex mixtures of unidentified compounds. With methyl iodide, however, it gave compound **3**, although to avoid the formation of, for instance, *C*-methylated side products (the formation of a product with a molecular weight of 442 has been demonstrated by DCI-mass spectrometry) the reaction time has to be kept relatively short; this results in relatively low product yields. Hydrogenation of the double bond of **3** in the presence of Pd–C yields compound **4** almost quantitatively, although, prolonged reaction times or large amounts of catalyst have to be avoided, since they result in ring opening of the dihydrofuran ring. $LiAlH_4$ reduction of both ester functions of **4** gave 3',4-di-*O*-methylcedrusin **5**.

The structures of compounds **2** to **5** were established on the basis of ^1H NMR-, ^{13}C NMR-, COSY, and HETCOR-spectral evidence (Tables 1 and 2).

Both natural 3',4-di-*O*-methylcedrusin and the racemic synthetic compound inhibit thymidine incorporation in endothelial cells. Of the compounds **2**–**5**, **5** was the most active.[2] In order to find out which enantiomers of 3',4-di-*O*-methylcedrusin are biologically active, pure enantiomers were needed. In an attempt to prepare these, compounds **2**–**5** were analysed by HPLC on several different chiral stationary phases (see Table 3). Compound **3** was sufficiently well resolved on Chiralcel OJ for use of the latter on a preparative scale. Although the resolution was better with ethanol (see Table 3), methanol (100%, $\alpha = 1.20$) was used as the eluent because compound **3** is more soluble in this solvent. The enantiomers of **4** and **5** have been synthesised from the enantiomers of **3** in the same way as the racemic compounds. The enantiomers **4a** and **5a** were prepared from the first eluted enantiomer **3a** and the antipodes **4b** and **5b** from the enantiomer **3b**.

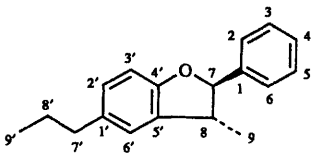


Scheme 1 *Reagents:* i, MeOH, DOWEX 50W × 8 200–400; ii, Ag_2O, acetone–benzene; iii, MeI, K_2CO_3, acetone; iv, H_2, Pd–C; v, $LiAlH_4$, THF–Et_2O

Absolute configurations were assigned to compounds **3b**,† **4b** and **5b** on the basis of UV and CD spectroscopic evidence. All have similar spectra except that for compound **3b**, in which the extended chromophore of one of the aromatic rings absorbs

† The spectrum shown in Fig. 1 is the inverted CD-spectrum of **3a**, since this was of better quality than the spectrum of **3b**.

Table 1 ^{1}H NMR spectra



Hydrogen atom[a]	Compd. **2**[b] δ_H/ppm	Mult.	J/Hz	Long range HETCOR[f]	Compd. **3**[g] δ_H/ppm	Mult.	J/Hz	Long range HETCOR[f]	Compd. **4**[g] δ_H/ppm	Mult.	J/Hz	Long range HETCOR[f]	Compd. **5**[i,j] δ_H/ppm	Mult.	J/Hz	Long range HETCOR[f]
2-H	7.09	d	2	C-3, C-4, C-6, C-7	6.92	d	1.99	C-1, C-3/4	6.95	d	1.83	C-3/4, C-6, C-7	6.95	m		C-3, C-4, C-7[k]
5-H	6.85	d	8	C-1, C-3	6.84	d	8.35	C-1, C-3/4	6.83	d	8.09	C-3/4, C-1	6.82	d	8.7	C-3, C-4
6-H	6.91	dd	2; 8	C-2, C-4, C-7	6.97	dd	8.35; 1.99	C-1, C-2, C-3/4, C-7	6.97	dd	1.99; 8.09		6.95	m		C-3, C-4, C-7[k]
7-H	6.03[c]	d	8[e]	C-1, C-2, C-6, C-8	6.13	d	8.15[e]		6.05	d	8.39[e]	C-9	5.54	d	7.17[e]	C-6, C-9
8-H	4.48[c]	d	8[e]	C-1, C-7, C-9, C-5′	4.35	d	8.15[e]	C-9, C-5′	4.31	d	8.39[e]	C-9, C-5′	3.59	m		C-1, C-5′
9-H	—				—				—				3.93	dd	10.8; 6.1	C-7, C-5′
													3.88	dd	10.8; 4.9	
2′-H	7.33	br[d]		C-3′, C-4′, C-6′, C-7′	7.03			C-3′, C-4′, C-6′, C-7′	6.70	br[d]		C-3′, C-4′, C-6′, C-7′	6.67[h]	br[d]		C-3′, C-4′, C-7′[l]
6′-H	7.30	br[d]		C-8, C-2′	7.19				6.80	br[d]		C-4′, C-6′	6.65[h]	br[d]		C-3′, C-4′, C-7′[l]
7′-H	7.63	d	16	C-2′, C-6′, C-8′, C-9′	7.65	d	15.91		2.91	t	±7.7	C-1′, C-6′, C-8′	2.66	br[d]t		C-1′, C-2′, C-8′, C-9′
8′-H	6.45	d	16	C-1′	6.32	d	15.91		2.62	t	±7.7	C-1′, C-7′, C-9′	1.87	m		C-1′, C-7′, C-9′
9′-H	—				—				—				3.67	t	6.34	C-7′, C-8′
3-OCH_3	3.83	s		C-3	3.86[h]	s			3.86	s		C-3, C-4	3.84	s		C-3
4-OCH_3	—				3.87[h]	s			3.86	s			3.85	s		C-4
9-OCH_3	3.82	s		C-9	3.84	s			3.81	s		C-9	—			
3′-OCH_3	3.93	s		C-3′	3.92	s			3.88	s		C-3′	3.88	s		C-3′
9′-OCH_3	3.73	s		C-9′	3.80	s			3.68	s		C-9′	—			

[a] The numbering used here is as shown in the structure at the top of this table and is used for easy comparison of the signals of the compounds **2**–**5**, but is different from the numbering in nomenclature. [b] Varian XL-300, 300 MHz, 60 mg/0.8 cm^3 [$^2H_6$]-acetone, room temperature, TMS. [c] Reassigned compared with ref. 8. [d] Broad. [e] This coupling constant can not be used for the assignment of a *trans*-configuration since $J_{cis} \approx J_{trans} \approx 8$ Hz.[11] [f] In a ^1J HETCOR spectrum coupling of all H-atoms with the corresponding C atoms is seen. [g] Varian Unity 400, 400 MHz, 20 mg/cm^3 $CDCl_3$, room temperature, TMS. [h] Assignments may be reversed. [i] Varian Unity 400, 400 MHz, 39 mg/0.8 cm^3 $CDCl_3$, room temperature, TMS. [j] δ_H-Values of **5** in CD_3OD have previously been published.[10] [k] Coupling of 2-H and 6-H with these C atoms cannot be distinguished. [l] Coupling of 2′-H and 6′-H with these C atoms cannot be distinguished.

Table 2 ^{13}C NMR spectra

Carbon atom[a]	Compd. 2[b]		Compd. 3[c]		Compd. 4[c]		Compd. 5[e,f]	
	δ_c/ppm	DEPT	δ_c/ppm	DEPT	δ_c/ppm	DEPT	δ_c/ppm	DEPT
C-1	131.81	C	132.18	C	133.04	C	133.92	C
C-2	110.96	CH	109.56	CH	110.25	CH	109.63	CH
C-3	148.75	C	149.49[d]	C	149.65	C	149.26	C
C-4	148.21	C	149.62[d]	C	149.65	C	149.07	C
C-5	116.02	CH	111.46	CH	111.90	CH	111.30	CH
C-6	120.18	CH	118.82	CH	118.83	CH	118.64	CH
C-7	88.41	CH	87.41	CH	86.72	CH	87.70	CH
C-8	55.92	CH	55.58	CH	56.23	CH	53.81	CH
C-9	171.73	C	170.74	C	171.24	C	64.04	CH_2
C-1′	129.42	C	128.76	C	134.37	C	135.37	C
C-2′	113.46	CH	112.53	CH	113.74	CH	112.78	CH
C-3′	145.80	C	144.85	C	144.50	C	144.19	C
C-4′	151.01	C	150.14	C	146.74	C	146.62	C
C-5′	127.35	C	125.88	C	125.51	C	127.90	C
C-6′	119.02	CH	117.99	CH	116.76	CH	116.11	CH
C-7′	145.50	CH	144.72	CH	36.17	CH_2	31.96	CH_2
C-8′	116.29	CH	115.76	CH	30.96	CH_2	34.55	CH_2
C-9′	167.86	C	167.55	C	173.16	C	62.22	CH_2
3-CH_3	56.35	CH_3	56.05[d]	CH_3	56.15[d]	CH_3	55.97	CH_3
4-CH_3	—		56.09[d]	CH_3	56.20[d]	CH_3	55.97	CH_3
9-CH_3	53.07	CH_3	52.83	CH_3	52.52	CH_3	—	
3′-CH_3	56.54	CH_3	56.29	CH_3	56.45	CH_3	56.12	CH_3
9′-CH_3	51.67	CH_3	51.58	CH_3	51.46	CH_3	—	

[a] The numbering used here is as shown in the structure at the top of Table 1 and is used for easy comparison of the signals of the compounds **2** to **5**, but is different from the numbering in nomenclature. [b] Varian XL-300, 75 MHz, 60 mg/0.8 cm^3 [$^2H_6$]acetone, room temp., TMS. [c] Varian Unity 400, 100 MHz, 20 mg/cm^3 $CDCl_3$, room temp., TMS. [d] Assigment may be reversed. [e] Varian Unity 400, 100 MHz, 39 mg/0.8 cm^3 $CDCl_3$, room temp., TMS. [f] δ_C-Values of **5** in CD_3OD have previously been published.[10]

Table 3 Separation factors (α) for chiral separations of compounds **2**–**5**

Compound	Chiralcel OD EtOH/C_6H_{14} 1:1	Chiralcel OD EtOH	Chiralpak AD EtOH	Chiralcel OJ EtOH
2	1.15	1.05	1.13	1.26
3	1.13	1.08	1.18	1.32
4	1.00	1.00	1.00	1.09
5	1.00	1.00	1.00	1.00

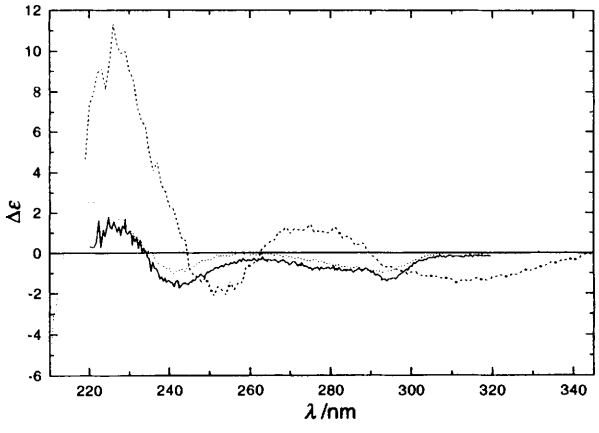
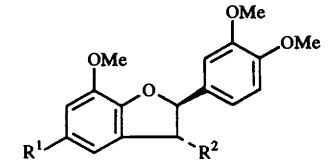


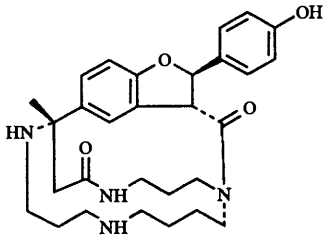
Fig. 1 CD spectra of compounds **3b**† - - -, **4b** . . ., and **5b** ——. [**3**] = 7.01 × 10^{-4}, [**4**] = 9.4 × 10^{-4} and [**5**] = 7.9 × 10^{-4} mol dm^{-3}, all in CH_2Cl_2.

† See footnote on p. 1775.

> 300 nm (UV 324 nm, ε 20 580; and CD: 312 nm, $\Delta\varepsilon$ −1.7, Fig. 1). This absorption which is absent for compounds **4b** and **5b** is quite probably the conjugated α-band of the dihydrobenzofuran aromatic ring. The very low chiral absorption of this band (*g ca.* 2 × 10^{-4}) implies that this is a chirally disturbed, not inherently chiral chromophore. Compounds **4** and **5** have UV maxima at 285 and 282 nm (ε 4900 and 8000, respectively); compound **3** can easily contain this band under the broad long wavelength absorption. Both compounds **4b** and **5b** have negative, though very low CD absorption corresponding to this band. All three compounds have a band with positive sign pattern in the 240–220 nm region (230 nm in **3b**, 220 nm in **4b** and 225 nm in **5b**) corresponding to the strong UV band at 230 nm. That the sign of this band is the same in all three compounds makes it probable that they have the same absolute configuration, which would be expected since compounds **4b** and **5b** were synthesised from compound **3b**. However, with $\Delta\varepsilon$ values of < 3 for most of the bands, the absolute configuration of the compounds cannot be predicted. Nevertheless, our CD spectra can be compared with the CD spectra[12–16] of the ephedradines A (orantine), B, C and D, and of *O*-methylorantine, which have a (2*R*,3*R*)-*trans*-substituted dihydrobenzofuran skeleton, as was determined by anomalous dispersion X-ray crystallography[12] of ephedradine

Table 4 Tentative configuration assignment





Reference compound ephedradine A[12]

Compound	R^1	R^2	Configuration
3b	$CH{=}CHCO_2Me$	CO_2Me	2*R*,3*R*
4b	$CH_2CH_2CO_2Me$	CO_2Me	2*R*,3*R*
5b	$CH_2CH_2CH_2OH$	CH_2OH	2*R*,3*S*‡

A. The CD spectra do not agree in the longwave region of the spectrum where the positive charge of the protonated ephedradines influences the spectrum. The 200–250 nm region, however, fits fairly well: a negative absorption at 210 nm, a positive band at 220 nm and a negative absorption at 240 nm (**4b**, Fig. 1). Because of this we tentatively assign a 2*R*,3*R*-configuration to compounds **3b** and **4b** and a 2*R*,3*S*‡-configuration to compound **5b**. A CD-spectrum of natural 3',4-di-*O*-methylcedrusin from *Sangre de drago* has been recorded. This is identical with the CD spectrum of **5b**, which means that a tentative 2*R*,3*S*-configuration can be assigned to the natural compound.

Experimental

Natural 3',4-di-*O*-methylcedrusin was isolated from *Sangre de drago* obtained from Peruvian *Croton spp.*[1] The molecular weights of compounds **2–5** were determined by DCI-mass spectrometry.

Methyl ferulate 1

A mixture of ferulic acid (4 g, 21 mmol), absolute methanol (25 cm^3) and Dowex 50 W × 8200–400 (0.4 g) was heated under reflux overnight after which it was filtered and evaporated under reduced pressure to afford the product (100%). This was used without further purification.

Methyl (*E*)-3-[2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoate 2

A two-necked flask (250 cm^3) covered with aluminum foil and equipped with a magnetic stirrer, a gas inlet tube (N_2) and a $CaCl_2$ tube, was charged with methyl ferulate (2.4 g, 11.5 mmol), silver oxide (1.4 g, 5.9 mmol), dry benzene (40 cm^3) and acetone (24 cm^3). After being flushed with N_2 gas for 5 mins, the flask was sealed, and the mixture stirred for 20 h at room temperature. The silver oxide was then filtered off and washed with ethyl acetate. The combined organic layers were evaporated under reduced pressure to afford a residual red-brown oil which was purified by column chromatography (column: 30 × 3.8 cm silica gel 60, 0.040–0.063 mm) with ethyl acetate–hexane (3:5) as eluent. This gave the title compound **2** as a white powder (751 mg, 31%), mp 151–152 °C.

Methyl (*E*)-3-[2-(3,4-dimethoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoate 3

Compound **2** (910 mg, 2.2 mmol) was stirred with acetone (20 cm^3) in a round-bottomed flask (250 cm^3), equipped with a reflux condenser and a $CaCl_2$-tube, until dissolution occurred. Anhydrous K_2CO_3 (5 g) and methyl iodide (15 cm^3) were then added to the mixture which was then heated under reflux for 22 h during which time it became bright yellow. After evaporation of the mixture under reduced pressure, the residue was treated in water (150 cm^3) and extracted with ethyl acetate (4 × 100 cm^3). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to afford a crude product which was purified by column chromatography (column: 30 × 3.8 cm silica gel 60, 0.040–0.063 mm) with ethyl acetate–hexane as eluent [300 cm^3 (4:1), 280 cm^3 (3.6:1) and 1000 cm^3 (2.8:1)]. This afforded the title compound **3** as a white powder (390 mg, 42%), mp 135–136 °C.

Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]propanoate 4

Compound **3** (366 mg, 0.85 mmol) and ethyl acetate (35 cm^3) were stirred for 5 min in a two-necked flask (100 cm^3) equipped with a magnetic stirrer, a gas inlet tube (N_2) and a $CaCl_2$ tube, flushed with N_2 gas. Pd/C (137 mg) was then added to the mixture and the flask flushed with H_2 gas. After the flask had been sealed the mixture was stirred for 40–60 mins at room temperature. The flask was then flushed again with N_2 gas and the catalyst filtered off and washed with ethyl acetate. The combined solutions were evaporated under reduced pressure to give a colourless oil which was dissolved in methanol (1–2 cm^3) and the solution kept at 18 °C until a white precipitate formed. The mixture was then evaporated under reduced pressure to afford the crude product (363 mg, 98.8%). Recrystallisation of this from methanol afforded the title compound **4** (280 mg; 76.5%) as a white powder, mp 97–99 °C.

3',4-Di-*O*-methylcedrusine, 3-[2-(3,4-dimethoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-propan-1-ol 5

A mixture of $LiAlH_4$ (4.26 mg, 11.2 mmol) and dry diethyl ether (30 cm^3) in a three-necked flask (100 cm^3) equipped with a magnetic stirrer, a dropping funnel, a gas inlet tube (N_2) and a $CaCl_2$ tube, was continuously stirred and flushed with N_2 gas while a solution of compound **4** (446 mg, 1.04 mmol) in dry THF (30 cm^3) was added dropwise over 15 min. After the addition, the mixture was flushed for a further 5 min with N_2 and then stirred for 4 h at room temperature. After the residual $LiAlH_4$ had been destroyed by adding water (5 cm^3) dropwise to the cooled mixture, concentrated HCl was also added dropwise until two clear layers formed. After separation, the aqueous layer was extracted with diethyl ether (6 × 50 cm^3) and the combined extracts were then dried ($MgSO_4$), filtered and evaporated at reduced pressure to afford a brown oil (362 mg, 93.4%). This was purified by column chromatography (column: 20 × 3.0 cm silica gel 60, 0.040–0.063 mm) with ethyl acetate–hexane as eluent [400 cm^3 (1:1); 500 cm^3 (1.5:1), 140 cm^3 (1.8:1), 300 cm^3 (2:1) and 375 cm^3 (4:1)] to afford the title compound **5** as a colourless oil (252.2 mg, 56%).

Preparative chiral HPLC

For analytical purposes the enantiomeric separation of compound **3** was easily achieved on a 3,5-dimethylphenyl-

‡ The inverse chirality label at C-3 is only the result of a different sequence of the substituents according to the Sequence Rule of the Cahn-Ingold-Prelog convention.[17]

cellulose carbamate column (Chiralcel OD; Daicel) using hexane–propan-2-ol (70:30 v/v) or hexane–ethanol (1:1 v/v; Table 3) as eluent. However, due to solubility problems in the mobile phase, an alternative approach was necessary for the preparative chromatographic separation. On a Chiralcel OJ column (*p*-methylbenzoyl cellulose; Daicel) using pure methanol as eluent a nearly baseline separation (Kaiser resolution: 0.91) could be achieved under the following experimental conditions: Prepbar 200 automated process chromatographic unit (Merck) with a stainless-steel column (100 mm ID × 500 mm) equipped with a water jacket and filled with Chiralcel OJ (*ca.* 2 kg). Methanol 150 cm^3 min^{-1} was used as the mobile phase, $T = 30$ °C, UV detection at 220 nm. Sample size 2.1 g dissolved in the eluent.

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