The Absolute Stereochemistries of (+)-Toddalolactone and its Related Chiral Coumarins from *Toddalia asiatica* (L.) Lam. (*T. aculeata* Pers.) and their Optical Purities

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The absolute stereochemistries of (+)-toddalolactone (+)-**1** and its related chiral coumarins [(+)-toddanol (+)-**2**, (+)-6-(2-hydroxy-3-methoxy-3-methylbutyl)-5,7-dimethoxycoumarin (+)-**3**, (+)-6-(3-chloro-2-hydroxy-3-methylbutyl)-5,7-dimethoxycoumarin (+)-**4** and (−)-aculeatin (−)-**5**], isolated from *Toddalia asiatica* (L.) Lam. (*T. aculeata* Pers.), have been determined as *R* by ozonolysis of (+)-**1** and by the chemical intercorrelation. Their optical purities were estimated by means of an ^{1}H NMR lanthanoid induced shift study using Eu(hfbc)$_3$.

The root bark of *Toddalia asiatica* (L.) Lam. (*T. aculeata* Perrs.) (Rutaceae) has been used as a folk medicine in India, China, Taiwan and Okinawa in Japan and had been also used in Europe under the name of Lopez root in 18th century.[1] It is well known that (+)-toddalolactone (+)-**1** is a main component of this plant.[2] Recently, we examined the chemical constituents of the root bark of this plant collected at Okinawa and Taiwan and reported the isolation of (+)-**1** together with its related chiral coumarins [(+)-toddanol (+)-**2**, (+)-6-(2-hydroxy-3-methoxy-3-methylbutyl)-5,7-dimethoxycoumarin (+)-**3** and (+)-6-(3-chloro-2-hydroxy-3-methylbutyl)-5,7-dimethoxycoumarin (+)-**4**] from the methanol extract[3] and the isolation of (−)-aculeatin (−)-**5** from the hexane or the carbon dioxide extracts.[4]

Although the plain structure of (+)-**1** was established to be 6-(2,3-dihydroxy-3-methybutyl)-5,7-dimethoxycoumarin by Späth *et al.*[5] *ca.* 50 years ago, the absolute configuration of the chiral centre in the side chain has remained unresolved. The absolute stereochemistries of other chiral coumarins have not been also determined yet. We succeeded in chemically confirming their stereochemistries to be *R* and found that these naturally occurring chiral coumarins were not enantiomerically pure. In this report we discuss their absolute stereochemistries and optical purities.

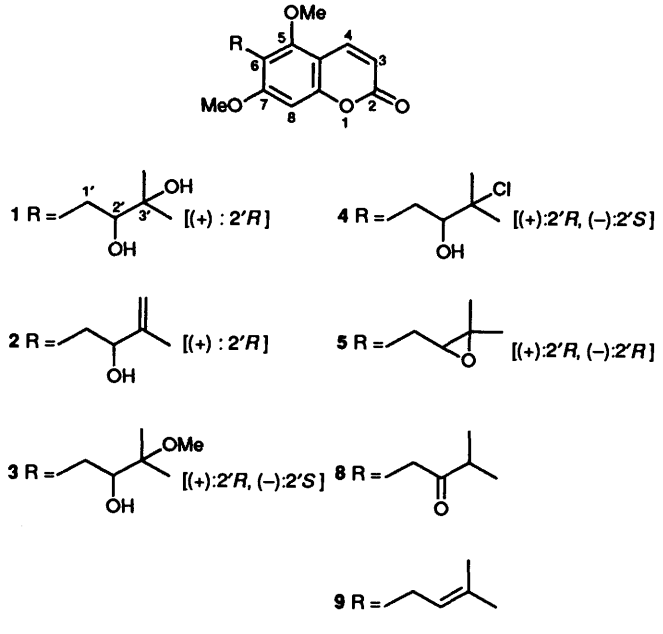
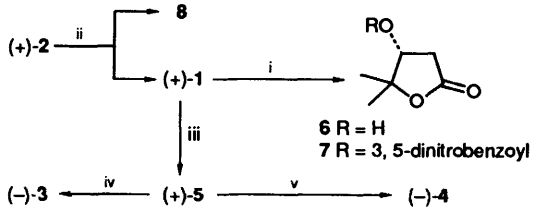


Chart 1

Results and Discussion

As reported previously compounds **1**, **2**, **3** and **4** were isolated as crystals having dextrorotatory specific rotations ([α]$_D$) {(+)-**1**: m.p. 134–140 °C, [α]$_D^{16}$ + 68.9† (*c* 0.616, in $CHCl_3$); (+)-**2**: m.p. 118–120 °C, [α]$_D^5$ + 51.0 (*c* 2.09, in $CHCl_3$); (+)-**3**: m.p. 113–117 °C, [α]$_D^{15}$ + 32.4 (*c* 0.079, in $CHCl_3$); (+)-**4**: m.p. 150–152 °C, [α]$_D^{20}$ + 73.8 (*c* 0.011, in $CHCl_3$)}[3], while crystalline **5** had a levorotatory [α]$_D$ {(−)-**5**, m.p. 77–83 (−105) °C, [α]$_D^{29}$ − 11.6 (*c* 0.5, in AcOEt)}[4] (Chart 1). The absolute stereochemistry of (+)-**1** was determined as *R* since on ozonolysis it gave the known (+)-(*R*)-lactone **6**[6], characterized as its 3,5-dinitrobenzoate **7**. The *R* configuration of (+)-**2** was established by its conversion with hot aqueous oxalic acid into (+)-**1** along with the isomerised toddanone **8**[3]. Retention of configuration is consistent with a transition state in which the positive charge developed on a tertiary carbon atom. According to an established chemical method[7] the (+)-epoxide (+)-**5**, m.p. 81–83 (−110) °C, [α]$_D^{15}$ +13.1, an enantiomer of a natural product, was afforded by treatment with triethylamine of the toluene-*p*-sulfonate of (+)-**1**. Since it was probable that the intramolecular nucleophilic displacement of the secondary tosyloxy group by the tertiary hydroxy group would proceed with inversion of configuration, synthetic (+)-**5** was assigned an *S* configuration. In other words, natural (−)-**5** has to be of *R* configuration. The *R* configurations of natural (+)-**3** and (+)-**4** were also assigned from preparation of the enantiomeric derivatives {(−)-**3**: m.p. 112–117 °C, [α]$_D^{14}$ −37.6; (−)-**4**: m.p. 150–152 °C, [α]$_D^{15}$ −73.5} by treatment of synthetic (+)-**5** with boron trifluoride in methanol and with ethereal hydrogen chloride, respectively (Scheme 1). These reactions too would be



Scheme 1 *Reagents and conditions:* i, O_3 then H_2O_2; ii, aq. $(CO_2H)_2$; iii, TsCl, pyridine then Et_3N; iv, BF_3-Et_2O, MeOH; v, HCl, Et_2O

expected to proceed with complete retention of configuration at the chiral centre. Thus, it was clear that the absolute stereochemistries of all the chiral coumarins isolated from *T. asiatica* (*T. aculeata*) should be assigned as *R*.

† [α]$_D$ Values are recorded in units of 10^{-1} deg cm^2 g^{-1}

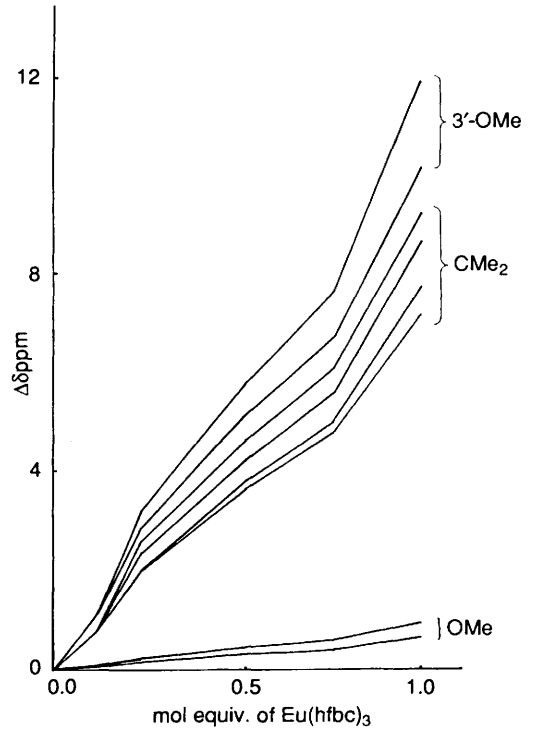


Fig. 1 Variation in the chemical shifts of the selected signals of (±)-**3** (1.7×10^{-3} mol dm^{-3}, in $CDCl_3$) with increasing concentration of $Eu(hfbc)_3$ in the 60 MHz ^1H NMR spectra

During the purification of (+)-**3** it was found to crystallize in three distinct forms. Thus, distillation of a chromatographically purified oily product afforded colourless prisms (cryst.-I), m.p. 90–112 °C, $[\alpha]_D^{35}$ +43.0, which upon recrystallization gave further crystalline form (cryst.-II), m.p. 92–94 °C, $[\alpha]_D^{35}$ +47.6. The third form (cryst.-III), m.p. 112–117 °C, $[\alpha]_D^{35}$ +32.0, was obtained from the mother liquor of cryst.-II. The IR spectra of these three forms showed non-identical absorption patterns in the solid state, but completely identical ones in the solution; this indicated that formation of the different forms depended on either optical purities or polymorphic crystallization. The $[\alpha]_D$ values strongly suggested that the former was the more responsible. Thus, we planned to estimate the enantiomeric purity of each form by an ^1H NMR lanthanoid induced shift (LIS) study using a chiral shift reagent.

Initially, racemic **3** was prepared for preliminary studies. Thus, successive treatment of toddaculin **9**[3] with *m*-chloroperbenzoic acid and methanolic boron trifluoride gave the desired product, *via* the epoxide (±)-**5**. The ^1H NMR spectra of (±)-**3** in deuteriochloroform upon stepwise addition of europium(III) tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate], $Eu(hfbc)_3$, showed effective deshieldings of signals assigned to aliphatic methoxy and *gem*-dimethyl groups with sufficient signal separation between the two enantiomers as a result of diastereoisomeric co-ordination of the shift reagent to the substrate (Fig. 1). Next, three kinds of crystals of (+)-**3** were subjected to the LIS study. The ^1H NMR spectrum of each in the presence of 1.5 mol equiv. of $Eu(hfbc)_3$ is given in Fig. 2. These disclosed that cryst.-II was optically pure, while cryst.-I and -III partially involved, respectively, *ca.* 5 and 20% of the (−)-enantiomer. Since cryst.-I, isolated from natural sources and purified without any recrystallization, contained both enantiomers we initiated an independent determination of the optical purity of a natural epoxide (−)-**5**.

In the previous paper,[4] we pointed out the possibility that ring-opened chiral coumarins such as (+)-**1** and (+)-**3** could be

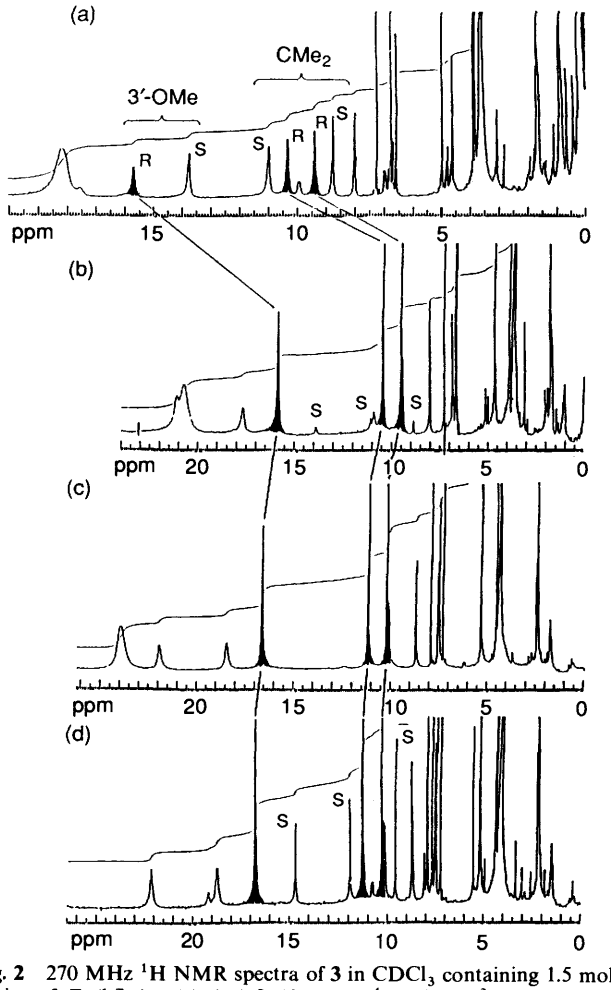


Fig. 2 270 MHz ^1H NMR spectra of **3** in $CDCl_3$ containing 1.5 mol equiv. of $Eu(hfbc)_3$: (*a*) (±)-**3** (6.2×10^{-4} mol dm^{-3}); (*b*) cryst.-I (5×10^{-4} mol dm^{-3}); (*c*) cryst.-II (5×10^{-4} mol dm^{-3}); (*d*) cryst.-III (4.5×10^{-4} mol dm^{-3})

artefacts derived from an epoxide (−)-**5** during isolation. If the conversion lacked complete stereospecificity, inversion of configuration could lead to the presence of a small proportion of an unexpected enantiomer. From a LIS study using racemic **5** the signals attributable to 4-H and the *gem*-dimethyl group were found to be the most diagnostic in this case. The ^1H NMR spectrum of chromatographically purified (−)-**5** containing 0.6 mol equiv.* of $Eu(hfbc)_3$ clearly showed the presence of the (+)-enantiomer at the same ratio that the (−)-enantiomer occurred in cryst.-I of (+)-**3** (Fig. 3); this indicated that (−)-**5**, a genuine natural product, did not occur in nature as a single enantiomer. Repeated recrystallization gave optically pure (−)-**5**, m.p. 81–84 °C, $[\alpha]_D^{23}$ −13.95.

Finally, both our sample and Späth's sample† of (+)-**1** were subjected to a LIS study. As expected, the study showed that the (−)-enantiomer was present in our sample. Interestingly, the same result was obtained for Spath's sample which had been isolated from the plant in India, a different region from ours. Optically pure (+)-**1**, m.p. 135.5–137 °C, $[\alpha]_D^{10}$ +74.5, was also provided by repeated recrystallization. Thus, it is reasonable to conclude that chiral coumarins of *T. asiatica* (*T. aculeata*) exist in the plant body as partial racemates and were isolated as racemic mixtures.

* Addition of more than 0.6 mol equiv. of $Eu(hfbc)_3$ caused collapse of signals.

† The sample was given by Prof. Silhan of Wien university for identification.

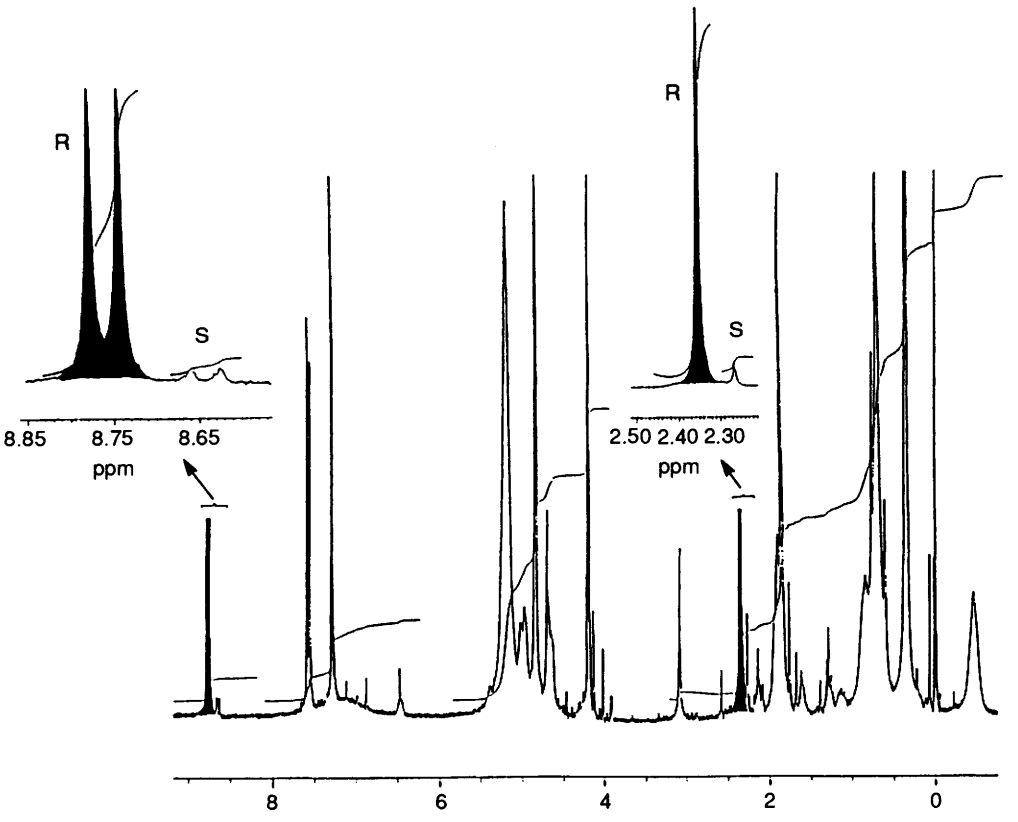


Fig. 3 270 MHz ^{1}H NMR spectrum of natural (−)-**5** (4.9×10^{-4} mol dm^{-3}, in $CDCl_3$) containing 0.61 mol equiv. of Eu(hfbc)$_3$

Although for the majority of naturally occurring optically active coumarins only one enantiomer is known, some examples[8] have been reported where both enantiomeric forms, one enantiomer and a racemate, and only the racemate have been isolated. Since no earlier discussion on the enantiomeric purities of such optically active coumarins by an unambiguous and systematic method has appeared, this report is the first. Moscher's method[9] using α-methoxy-α-(trifluoromethyl)-phenylacetic acid has been recently developed for determination of enantiomeric purity by means of ^{1}H NMR spectroscopy. Nevertheless, the LIS method can still provide useful information because of the limited application of Moscher's method to alcohols and amines.

Experimental

All m.p.s were measured on a micro melting-point hot stage (Yanagimoto) and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ solution with JEOL FX- or GX-270 (270 MHz) or Hitachi R-24B (60 MHz) spectrometers, unless otherwise stated, with tetramethylsilane as internal reference. $[\alpha]_D$ and ORD were measured with JASCO DIP-140 and J-20 polarimeters using a 1 cm path cell, respectively and the values are given in 10^{-1} deg cm^2 g^{-1}. For column and flash chromatography, silica gel 60 (70–230 mesh ASTM; Merck) and silica gel 60 (230–400 mesh ASTM; Merck) were used, while for TLC and preparative TLC (PLC), DC-Fertigplatten SIL-G 25 UV254 (Macherey-Nagel) and silica gel GF_{254} (Merck) were used. A shift reagent, Eu(hfbc)$_3$, was purchased from Aldrich Chemical Co., Inc. and a calculated amount of it was directly added to a solution of a coumarin in $CDCl_3$ in NMR tube under argon.

Ozonolysis of (+)-*Toddalolactone* (+)-**1**.—(+)-(R)-*Dihydro-4-hydroxy-5,5-dimethylfuran-2*(3H)-*one* **6**.—According to the reported procedure[6b] (+)-**1** (2.01 g, 6.50 mmol) in dry chloroform (100 cm^3) was subjected to ozonolysis for 2.2 h. After work-up, column chromatography of the residue with diethyl ether followed by distillation at 90–100 °C/1 mmHg gave the known (+)-(*R*)-**6**[6] as a colourless oil (0.139 g, 16.4%); $[\Phi]_{500}$ +9.7 (*c* 1.04 × 10^{-2}, $CHCl_3$), $[\Phi]_{400}$ +27.0 and $[\Phi]_{300}$ +92.7.

3,5-*Dinitrobenzoate* **7**. Treatment of **6** (0.083 g, 0.64 mmol) in pyridine (2.3 cm^3, 28.4 mmol) with 3,5-dinitrobenzoyl chloride (0.354 g, 1.53 mmol) in dry benzene (1.4 cm^3) at 70 °C for 2 min afforded **7** as colourless needles (0.136 g, 66.1%) (from benzene–diethyl ether), m.p. 136–137 °C (lit.,[6b] m.p. 150 °C) (Found: C, 48.2; H, 3.7; N, 8.6. Calc. for: C, 48.15; H, 3.7; N, 8.6).

Treatment of (+)-*Toddanol* (+)-**2** *with Aqueous Oxalic Acid*.—A mixture of (+)-**2** (0.170 g, 0.59 mmol) in saturated aqueous oxalic acid (10 cm^3) and dioxane (10 cm^3) was heated at 120–150 °C for 36 h. After work-up flash chromatography of the residue with hexane–ethyl acetate (2:1) and then with ethyl acetate gave two fractions [Fr. 1 (0.044 g) and Fr. 2 (0.048 g)].

(*i*) *Toddanone* **8**: Recrystallization of Fr. 1 from chloroform–diethyl ether gave **8**[3] as colourless prisms (0.024 g, 14.1%), m.p. 115–120 °C.

(*ii*) (+)-*Toddalolactone* (+)-**1**: Recrystallization of Fr.2 from benzene–diethyl ether gave (+)-**1**[3] as colourless prisms (0.027 g, 14.8%), m.p. 130–135 °C; $[\alpha]_D^{20}$ +74.5 (*c* 0.082, in $CHCl_3$).

Transformation of (+)-*Toddalolactone* (+)-**1** *into* (+)-*Aculeatin* (+)-**5**.—Compound (+)-**1** (0.205 g, 0.66 mmol) was treated with toluene-*p*-sulfonyl chloride (0.403 g, 2.1 mmol) in dry pyridine (3.2 cm^3) at 5 °C for 2 weeks. After work-up the crude sulfonate (0.301 g) was dissolved in methylene dichloride (3.2 cm^3) and triethylamine (1.6 cm^3) and then heated at 35 °C for 1 week. After work-up PLC of the residue with choroform–ethyl acetate (20:1) afforded (+)-**5** as colourless prisms (0.147

g, 76.3%) (from ethyl acetate–hexane), m.p. 81–83 (−110) °C (Found: C, 66.0; H, 6.2. Calc. for $C_{16}H_{18}O_5$: C, 66.2; H, 6.25); $[\alpha]_D^{15}$ +13.1 (*c* 1.049, in AcOEt).

(−)-6-(2-*Hydroxy*-3-*methoxy*-3-*methylbutyl*)-5,7-*dimethoxycoumarin* (−)-**3**.—A solution of (+)-**5** (0.207 g, 0.71 mmol) in dry methanol (5 cm^3) was treated with boron trifluoride–diethyl ether (47%, 0.05 ml, 0.19 mmol) at room temperature for 30 min. After work-up, PLC of the residue with ethyl acetate–hexane (1:1) afforded (−)-**3** as colourless prisms (0.187 g, 81.3%) (from ethyl acetate–hexane), m.p. 112–117 °C (Found: C, 63.3; H, 6.8. Calc. for $C_{17}H_{22}O_6$: C, 63.3; H, 6.9); $[\alpha]_D^{14}$ −37.6 (*c* 0.611, in $CHCl_3$).

(−)-6-(3-*Chloro*-2-*hydroxy*-3-*methylbutyl*)-5,7-*dimethoxycoumarin* (−)-**4**.—A solution of (+)-**5** (0.311 g, 1.07 mmol) in dry diethyl ether (25 cm^3) was treated with dry diethyl ether (5 cm^3) saturated with hydrogen chloride under ice-cooling for 15 min. After work-up PLC of the residue with ethyl acetate–hexane (1:2) afforded (−)-**4** as colourless rods (0.188 g, 53.7%) (from diethyl ether), m.p. 150–152 °C (Found: C, 58.8; H, 5.8. Calc. for $C_{16}H_{19}ClO_5$: C, 58.8; H, 5.9); $[\alpha]_D^{15}$ −73.5 (*c* 0.802, in $CHCl_3$).

Purification of (+)-6-(2-*Hydroxy*-3-*methoxy*-3-*methylbutyl*)-5,7-*dimethoxycoumarin* (+)-**3**.—A chromatographically purified oily (+)-**3** (0.182 g) was distilled at 250 °C/0.1 mmHg to give a colourless oil (0.163 g), which was triturated with diethyl ether to afford cryst.-I as colourless prisms, m.p. 90–112 °C, $[\alpha]_D^{35}$ +43.0 (*c* 0.211, in $CHCl_3$). Recrystallization of it (0.131 g) from diethyl ether–hexane afforded cryst.-II as colourless prisms (0.053 g), m.p. 92–94 °C (Found: C, 63.4; H, 6.9. Calc. for $C_{17}H_{22}O_6$: C, 63.3; H, 6.9); $[\alpha]_D^{35}$ +47.6 (*c* 0.294, in $CHCl_3$). The mother-liquor of the recrystallization was then evaporated to dryness. Recrystallization of the residue from diethyl ether–hexane afforded cryst.-III as colourless scales (0.020 g), m.p. 112–117 °C (Found: C, 63.3; H, 6.85. Calc. for $C_{17}H_{22}O_6$: C, 63.3; H, 6.9); $[\alpha]_D^{35}$ +32.0 (*c* 0.115, in $CHCl_3$).

(±)-*Aculeatin* (±)-**5**.—A mixture of toddaculin **9**[3] (1.01 g, 3.67 mmol) and *m*-chloroperbenzoic acid (0.764 g, 4.43 mmol) in chloroform (10 cm^3) was stirred at room temperature for 10 min. After work-up, column chromatography of the residue with benzene–ethyl acetate (5:1) gave (±)-**5** as colourless prisms (0.879 g, 82.4%) (from ethyl acetate–hexane), m.p. 115–116 °C (Found: C, 66.2; H, 6.3. Calc. for $C_{16}H_{18}O_5$: C, 66.2; H, 6.25).

(±)-6-(2-*Hydroxy*-3-*methoxy*-3-*methylbutyl*)-5,7-*dimethoxycoumarin* (±)-**3**.—The same treatment of (±)-**5** (0.505 g, 1.74 mmol) in dry methanol (12 cm^3) with boron trifluoride–diethyl ether (47%, 0.12 cm^3, 0.45 mmol) as described above gave (±)-**3** as colourless scales (0.465 g, 82.9%), m.p. 121–124 °C (Found: C, 63.4; H, 6.8. Calc. for $C_{17}H_{22}O_6$: C, 63.3; H, 6.9).

Optically Pure (−)-(*R*)-*Aculeatin* (−)-**5**.—Repeated recrystallization of (−)-**5**,[4] m.p. 77–83 (−105) °C, $[\alpha]_D^{29}$ −11.6 (*c* 0.5, in AcOEt), from diethyl ether–isopropyl ether afforded optically pure (−)-(*R*)-**5** as colourless prisms, m.p. 81–84 °C (Found: C, 66.2; H, 6.3. Calc. for $C_{16}H_{18}O_5$: C, 66.2; H, 6.25); $[\alpha]_D^{23}$ −13.95 (*c* 0.57, in AcOEt).

Optically Pure (+)-(*R*)-*Toddalolactone* (+)-**1**.—Repeated recrystallization of (+)-**1**,[3] m.p. 134–140 °C, $[\alpha]_D^{16}$ +68.9 (*c* 0.616, in $CHCl_3$), from ethyl acetate afforded optically pure (+)-(*R*)-**1** as colourless prisms, m.p. 135–137 °C (Found: C, 62.4; H, 6.5. Calc. for $C_{16}H_{20}O_6$: C, 62.3; H, 6.5); $[\alpha]_D^{10}$ +74.5 (*c* 2.675, in $CHCl_3$).

Acknowledgements

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