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Isolation, Structure Determination and Synthesis of New Dihydroisocoumarins from *Ginkgo biloba* L.

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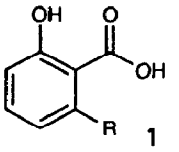
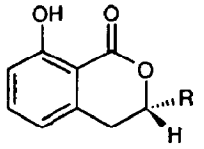
Abstract: New optically active 8-hydroxy-3-alk(en)yl-3,4-dihydroisocoumarins **2a-c** were isolated from *Ginkgo biloba* L. fruits. The absolute configuration was determined to be R by comparison with both enantiomers of the 8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin synthesized from optically active epichlorhydrins.

The ginkgo tree (*Ginkgo biloba* L.) is a "living fossil" and the only remaining member of the Ginkgoaceae family[1]. Male and female flowers are born on different plants and female plants bear a yellowish-green plum-like "fruit" (aril); the outer fleshy layer is malodorous and inedible. It surrounds an ovoid nut which has a sweet taste when roasted. Allergic contact dermatitis (ACD) to *Ginkgo biloba* L. occurs chiefly in eastern Asians who are aware of the culinary qualities of the nuts. However, in 1965, a small epidemic occurred in 35 American schoolgirls who trampled the fallen fruit[2]. A similar mini-epidemic was reported in France in 1988 among children who played marbles with the unripe fruit[3]. The main allergens from *Ginkgo biloba* L. were identified[4] as ginkgolic or anacardic acids **1**, a mixture of salicylic acid derivatives with long alkyl chains of 13 to 19 carbon atoms, fully saturated or with one or two double bonds.



1

R = $C_{13}H_{27}$
= $C_{15}H_{31}$, $C_{15}H_{29}$
= $C_{17}H_{35}$, $C_{17}H_{33}$, $C_{17}H_{31}$
= $C_{19}H_{37}$



2a R = $C_{13}H_{27}$
2b R = $C_{15}H_{29}$
2c R = $C_{17}H_{31}$

In the course of our research on the allergenic principles in *Ginkgo biloba* L. fruits, we have been interested in a mixture of non-polar compounds, present in trace amounts, and exhibiting a characteristic UV absorption. These coumponds were identified as members of a family of new optically active 8-hydroxy-3-alkyl-3,4-dihydroisocoumarins **2** probably derived from ginkgolic acids.

Isolation[5] and structure determination[6]. An extract of the fruit from *Ginkgo biloba* L., prepared by mechanical extraction in hexane, was initially chromatographed on silica and the least polar fractions were fractionated on Sephadex LH-20. The mixture of dihydroisocoumarins was then obtained by preparative chromatography on a silica plate. The gas chromatogram of the isolated product showed three peaks, which, by their different retention times, bring to mind a mixture of products of increasing chain length, as in the case of the ginkgolic acids. The mass spectra coupled to GC confirmed this hypothesis, as the mass increase between each peak was 28, i.e. two methylene groups. The base peak (100%), formed on removal of the side chain, indicated a mass of 163 for the part common to all the molecules. The infra-red spectrum of the mixture gave little information, apart from the presence of a phenol and of a carbonyl group at 1675 cm-1. Proton NMR spectra confirmed the presence of long chains and the signals corresponding to the aromatic part of the molecule showed the presence of 3 adjacent protons at 7.40 ppm (dd, J = 8.4 Hz, J = 7.1 Hz), 6.88 ppm (d, J = 8.4 Hz) and 6.68 ppm (d, J = 7.1 Hz). The presence of a phenolic proton strongly bound in a hydrogen bond at 11.03 ppm and of a benzylic methylene at 2.92 ppm (d, J = 7.0 Hz) coupled to a high multiplicity proton on

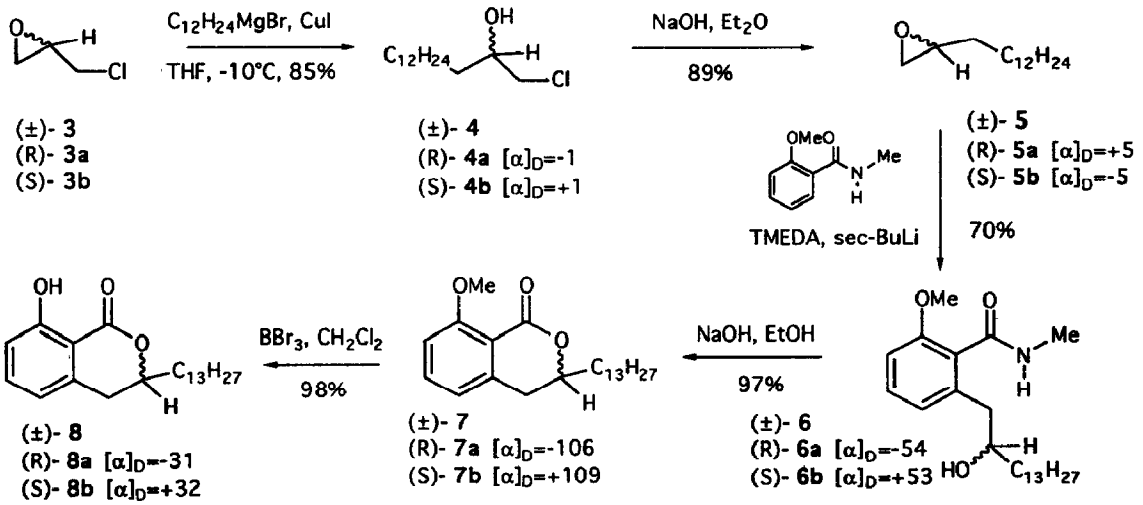
oxygen could also be seen. The most probable structure for the central part of the molecule was that of a dihydroisocoumarin nucleus. This is a family of natural products containing few representatives. Nevertheless, an analogous compound, with a side chain of 11 carbons, was isolated in 1980 from a brown alga *Caulocytis cephalornithos*.[7] The comparison of the ^{13}C NMR spectra confirmed the dihydroisocoumarin structure of our compounds.

Separation of the dihydroisocoumarin mixture by chromatography on preparative plates impregnated with silver nitrate gave three fractions consisting of saturated (the least polar), mono-unsaturated and di-unsaturated derivatives, with the mono-unsaturated fraction accounting for 85% of the total. GC-mass analysis of these three fractions showed that the saturated fraction consisted almost exclusively of the 13 carbon side chain derivative (m/z = 346) with a minute trace of the saturated 15 carbon side chain derivative (m/z = 374). The second fraction contained mainly (99%) the derivative with a 15 carbon mono-unsaturated side chain (m/z = 372) and 0.6% of a 17 carbon chain derivative (m/z = 400). Traces of the mono-unsaturated derivative with a 17 carbon chain were also detected. The third fraction contained a derivative with a 17 carbon di-unsaturated side chain (m/z = 398).

The mono-unsaturated fraction had an $[\alpha]^{25}_D = -23$ (c = 0.8, $CHCl_3$), which suggests the presence of an optically active center at the junction of the carbon chain and the dihydroisocoumarin system. The circular dichroism spectrum showed a negative maximum at 253 nm $[\theta]_{253} = -13000$ (c = 6.0 mM, $CHCl_3$), in perfect agreement with the UV absorption spectrum. By comparison with the dihydroisocoumarins described in the literature[8], it seems that optical rotation and negative circular dichroism are generally associated with an absolute configuration which would be R in our case. However, the very clear difference in the structure of the side chain and the fact that no optical characteristics have been described for the analogue isolated from *Caulocytis cephalornithos* means that this attribution could only be suggested.

The position of the double bond in the mono-unsaturated derivative was confirmed by mass spectrometry after oxidative cleavage by a catalytic amount of osmium tetroxide and an excess of sodium periodate[9]. This method has the advantage of allowing accurate quantification of the different reagents and of being able to stop the reaction with aldehyde. From the molecular ion peak at m/z = 262, the compound was determined to be the 8-hydroxy-3-(6'-pentadecenyl)-3,4-dihydroisocoumarin. This is in full agreement with the position of the double bonds in the ginkgolic acids[10] which could be the precursors of the coumarins.

Synthesis. To confirm the absolute conformation of the dihydroisocoumarins isolated from *Ginkgo biloba* L. and to have enough material for biological studies, we have synthesized the two enantiomers of the derivative with a saturated alkyl chain of 13 carbons **8a** and **8b**.



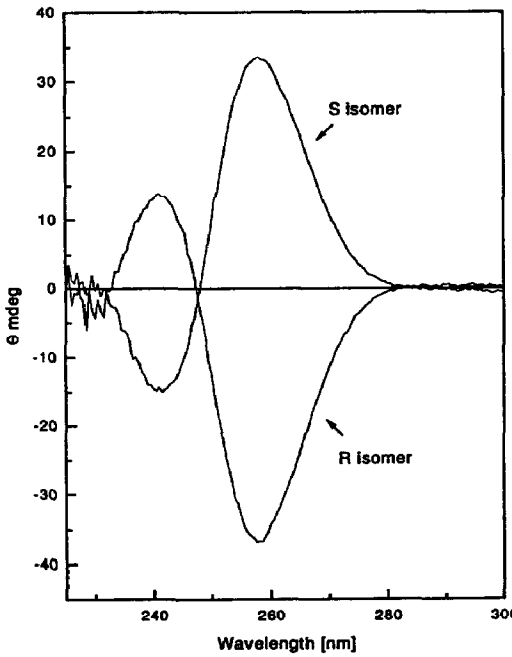
To unambiguously establish the absolute conformation, we started from chiral synthons of known absolute configuration[11]. The synthesis of aromatic derivatives by ortho-metalation has been well-studied in recent years and has proved effective in many cases[12]. Access to long-chain dihydroisocoumarins should

therefore be possible starting from an aromatic lithiated synthon and a chiral epoxide, the latter being prepared from two epichlorhydrins of R and S configurations.

The reaction of (R)-(-)- or (S)-(+)-epichlorhydrin with magnesium dodecyl bromide in the presence of CuI[13] gave, respectively, the (R)-(-)- and (S)-(+)-1-chloro-2-pentadecanol with a yield of 85%; these were converted into epoxides[14] by basic treatment (NaOH in ether) with a yield of 89%. Optical rotations conform, both in sign and absolute value, to those seen in the literature for analogous compounds[15].

The opening of (R)-(-)-1-chloro-2-pentadecanol or (S)-(+)-1-chloro-2-pentadecanol by the product of the ortholithiation of N-methyl-2-methoxybenzamide[16] produced, respectively, the alcohols **6a** and **6b** with yields of the order of 70%. It should be noted that the same reaction starting from the products of the ortholithiation of N,N-diethylmethoxybenzamide or the corresponding oxazoline did not give satisfactory results. The amide function was then hydrolysed in basic medium to yield spontaneously, after neutralisation, the (R)-(-)- and (S)-(+)-8-methoxy-3-tridecyl-3,4-dihydroisocoumarins[17] **7a** and **7b** which were then deprotected, with a yield of 98%, by treatment at -78°C with BBr_3[18] in CH_2Cl_2 to give the dihydroisocoumarins[19] **8a** and **8b**.

The spectroscopic characteristics of the synthetic 8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin were completely identical to those of product **2a** extracted from *Ginkgo biloba* L.. (R)-(-)-8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin **8a**, produced from (R)-(-)-epichlorhydrin **3a**, had an $[\alpha]^{23}_D$ of -31 (c = 1.0, $CHCl_3$), confirming the attribution of the R absolute configuration to the natural product. This attribution was also confirmed by the circular dichroism curves[20] of the two isomers (R)-(-)- and (S)-(+)-8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin compared with that of product **2b**. All the synthesized compounds had optical rotations of analogous value and opposing sign.



The 3,4-dihydroisocoumarins constitute a family, small in number. Recently, a group of molecules derived from 6,8-dihydroxy-3-undecyl-3,4-dihydroisocoumarin has been extracted from *Ononis natrix* L[21]. It should be noted that these molecules are also of the R configuration and have an $[\alpha]_D$ = -18. To our best knowledge, the only description of a long-chain 8-hydroxy-3-alkyl-3,4-dihydroisocoumarin is that of the 11-carbon derivative isolated from the brown alga *Caulocytis cephalornithos*.[7]

Little is known about the biological activities of the dihydroisocoumarins. Hydrangenol has been reported to be allergenic[22], A1-77-B to be gastroprotective[23] and 8-hydroxy-3-undecyl-3,4-dihydroisocoumarin to have anti-inflammatory properties. Having these molecules in large quantities and in the R and S forms will permit their allergizing properties to be tested and the stereospecificity of this immune reaction to be studied in more detail[24].

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5. ***Extraction and Isolation*** A crude extract of *Ginkgo biloba* L. fruits, collected in the Tokushima area (Japan), was prepared by mechanical extraction, using n-hexane as solvant. 250 g of this crude extract were chromatographed on silica gel using a gradient of hexane/ethyl acetate/methanol and collecting 10 fractions. Fractions 2 and 3 were then purified by chromatography on Sephadex LH-20 using chloroform-methanol (1:1) as eluent. Fractions containing non-polar compounds were futher purified by column chromatography on silica using a slow hexane/toluene gradient. 20 mg of a mixture of dihydroisocoumarins were finaly obtained by preparative TLC using an hexane/ethyl acetate (40:1) mixture as eluent. Saturated, mono-unsaturated and di-unsaturated dihydroisocoumarins were separated by preparative TLC on silica impregnated with silver nitrate. The elution solvent was hexane/ethyl acetate (9:1).
6. **(3R)-(-)-8-hydroxy-3-(6'-pentadecenyl)-3,4-dihydroisocoumarin 2b**: ^{1}H NMR (90 MHz) δ 0.88 (t, 3H, J = 7.0 Hz), 1.25 (bs, 18H), 2.02 (m, 2H), 1.75 (m, 2H), 2.92 (d, 2H, J = 7.0 Hz), 4.58 (m, 1H), 5.35 (m, 2H), 6.68 (d, 1H, J = 7.0 Hz), 6.88 (d,

1H, J = 8.4 Hz), 7.40 (dd, 1H, J = 7.0, J = 8.4 Hz), 11.03 (s, 1H). ^{13}C NMR δ 14.1, 22.7, 24.9, 27.2, 29.0, 29.2, 29.4, 29.7, 31.8, 31.9, 33.0, 34.8, 79.8, 108.6, 116.2, 117.9, 129.7, 130.0, 136.1, 139.5, 162.2, 169.9. IR (CCl_4) cm^{-1} 1675 (C=O). UV (MeOH) 219 (ε = 33500), 253 (ε = 21900), 261 (ε = 24200), 313 (ε = 3400). MS (relative intensity) m/z = 372 (35), 354 (13), 163 (100). $[\alpha]^{25}_D$ = -23 (c = 0.8, $CHCl_3$).

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17 **(±)-8-methoxy-3-tridecyl-3,4-dihydroisocoumarin 7:** white cristals: mp 73-74°C. ^1H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, 3H, J= 6.7 Hz, CH_3), 1.26-1.87 (m, 24H, CH_2), 2.86 (AB part of an ABX system, 2H, $\Delta\nu_{AB}$ = 12.7 Hz, J_{AB} = 16.1 Hz J_{AX} = 11.5 Hz, J_{BX} = 2.4 Hz, $ArCH_2$), 3.94 (s, 3H, OCH_3), 4.36 (X part of an ABX system, 1H, $ArCH_2CH$), 6.79 (d, 1H, J= 7.4 Hz, H_5), 6.91 (d, 1H, J= 8.4 Hz, H_7), 7.44 (dd like, 1H, J= 8.1 Hz, H_6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.8, 161.2, 142.1, 134.4, 119.2, 114.0, 110.9, 77.9, 56.2, 34.7, 34.5, 32.0, 29.7, 29.4, 25.0, 22.7, 14.1. IR (CCl4) cm^{-1} 1738 (C=O). Anal. calcd for $C_{23}H_{36}O_3$. C, 76,62; H, 10.06. Found: C, 76,46; H, 10.04.
(3R)-(-)-8-methoxy-3-tridecyl-3,4-dihydroisocoumarin 7a: Same procedure as for the racemic compound starting from **6a** (0.3 g; 0.76 mmol). White cristals: mp 77-78°C. $[\alpha]^{23}_D$ = -106 (c = 1, $CHCl_3$). Anal. calcd for $C_{23}H_{36}O_3$. C, 76,62; H, 10.06. Found: C, 76,83; H, 10.25.
(3S)-(+)-8-methoxy-3-tridecyl-3,4-dihydroisocoumarin 7b: Same procedure as for the racemic compound starting from **6b** (0.3 g; 0.76 mmol). White cristals: mp 77-78°C. $[\alpha]^{23}_D$ = +109 (c = 1, $CHCl_3$). Anal. calcd for $C_{23}H_{36}O_3$. C, 76,62; H, 10.06. Found: C, 76,85; H, 10.25.
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19 **8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin 8:** White cristals: mp 79-80°C. ^1H NMR (200 MHz, $CDCl_3$) δ, 0.88 (t, 3H, J= 6.7 Hz, CH_3), 1.26-1.91 (m, 24H, CH_2), 2.91 (AB part of an ABX system, 2H, $\Delta\nu_{AB}$ = 4.4 Hz, J_{AB} = 16.3 Hz, J_{AX} = 8.4 Hz, J_{BX} = 0.4 Hz, $ArCH_2$), 4.57 (X part of an ABX system, 1H, $ArCH_2CH$), 6.69 (d, 1H, J= 7.3 Hz, H_5), 6.88 (d, 1H, J= 8.3 Hz, H_7), 7.40 (dd like, 1H, J= 8.0 Hz, H_6), 11.03 (s, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.0, 162.2, 139.6,117.9, 116.2, 108.6, 79.8, 34.8, 33.0, 32.0, 29.7, 29.4, 24.9, 22.7, 14.2. IR (CCl_4) cm^{-1} 3428 (OH); 1684 (C=O). Anal. calcd.for $C_{22}H_{34}O_3$ C,76.26; H, 9.88. Found, C, 76.21; H, 9.92.
(3R)-(-)-8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin 8a: White cristals: mp 91-92°C. $[\alpha]^{23}_D$ = -31 (c = 1, $CHCl_3$). Anal. calcd for $C_{22}H_{34}O_3$ C,76.26; H, 9.88. Found, C, 76.47; H, 10.12.
(3S)-(+)-8-hydroxy-3-tridecyl-3,4-dihydroisocoumarin 8b: White cristals: mp 90-91°C. $[\alpha]^{23}_D$ = +32 (c = 1, $CHCl_3$). Anal. calcd for $C_{22}H_{34}O_3$ C,76.26; H, 9.88. Found, C, 76.62; H, 10.12.
20 CD spectra of **8a** and **8b** (c = 1.61 mM, $CHCl_3$, 25°C), step = 0.5 nm, averaging time = 3s, 3 repeats.
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