SYNTHESIS AND STRUCTURAL ELUCIDATION OF XANTHONOLIGNOIDS: *trans*-(±)-KIELCORIN B AND *trans*-(±)-ISOKIELCORIN B

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Abstract - This paper reports the synthesis and characterization of two isomeric xanthonolignoids - *trans*-(±)-kielcorin B and *trans*-(±)-isokielcorin B. The synthetic approach is based on the oxidative coupling of 2,3-dihydroxy-4-methoxyxanthone with coniferyl alcohol in the presence of silver carbonate. The structural elucidation of these compounds was achieved by extensive NMR studies: ^{1}H and ^{13}C NMR spectra, homonuclear correlation spectroscopy (COSY, COSYLR), heteronuclear correlation spectroscopy (HETCOR), nuclear Overhauser effect (NOE) and one-dimensional selective INEPT. The NMR spectroscopic techniques used in this study led to an unambiguous characterisation of *trans*-(±)-kielcorin B and *trans*-(±)-isokielcorin B, which includes the assignment of all proton and carbon resonances and the establishment of the substituents orientation and protons configurational relationship on their 1,4-dioxane ring.

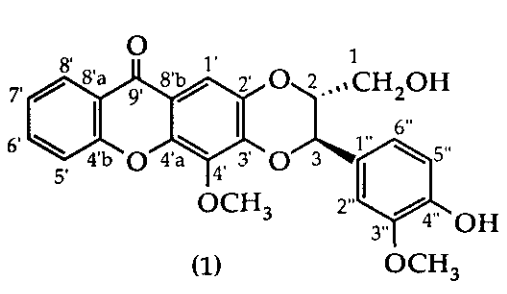
INTRODUCTION

trans-(±)-Kielcorin B (**1**) (Figure 1) is a naturally occurring xanthonolignoid, previously referred from *Kielmeyera coriacea*,[1] whose structure was tentatively elucidated by comparing its spectral data with those of kielcorin (**3**)[1-3] (Figure 1). Since *trans*-(±)-kielcorin B (**1**) was isolated in a very small quantity and contaminated with *trans*-(±)-kielcorin, it was not possible to determine its structure in an unequivocal way by chemical and spectroscopic methods.[1]

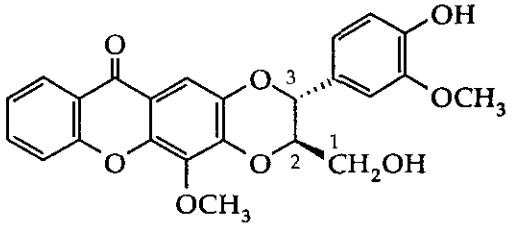
Although some lignoid type compounds have potent antihepatotoxic activity, *e. g.* silybin (which is already in the drug market for the treatment and prevention of liver diseases),[4]

little is known about the biological activity of xanthonolignoids. On the other hand, kielcorin and other xanthonolignoids have been found to induce a low cytotoxic activity,[5] This finding can be very important for the potential pharmacological use of these compounds.

The need to elucidate unequivocally the structure of kielcorin B by NMR techniques, combined with our interest in the evaluation of the antihepatotoxic activity of xanthonolignoids, has prompted us to elaborate a synthesis of *trans*-(±)-kielcorin B (**1**) and *trans*-(±)-isokielcorin B (**2**). A carefull search in the literature revealed the absence of reports for the synthesis of *trans*-(±)-kielcorin B (**1**) as well as for its isomer *trans*-(±)-isokielcorin B (**2**) which has never been reported as a natural product so far. Meanwhile the evaluation of the hepatoprotective activity of these compounds has been done and the results were published.[6]



(1)



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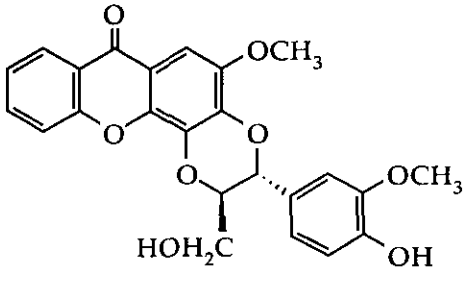


(3)

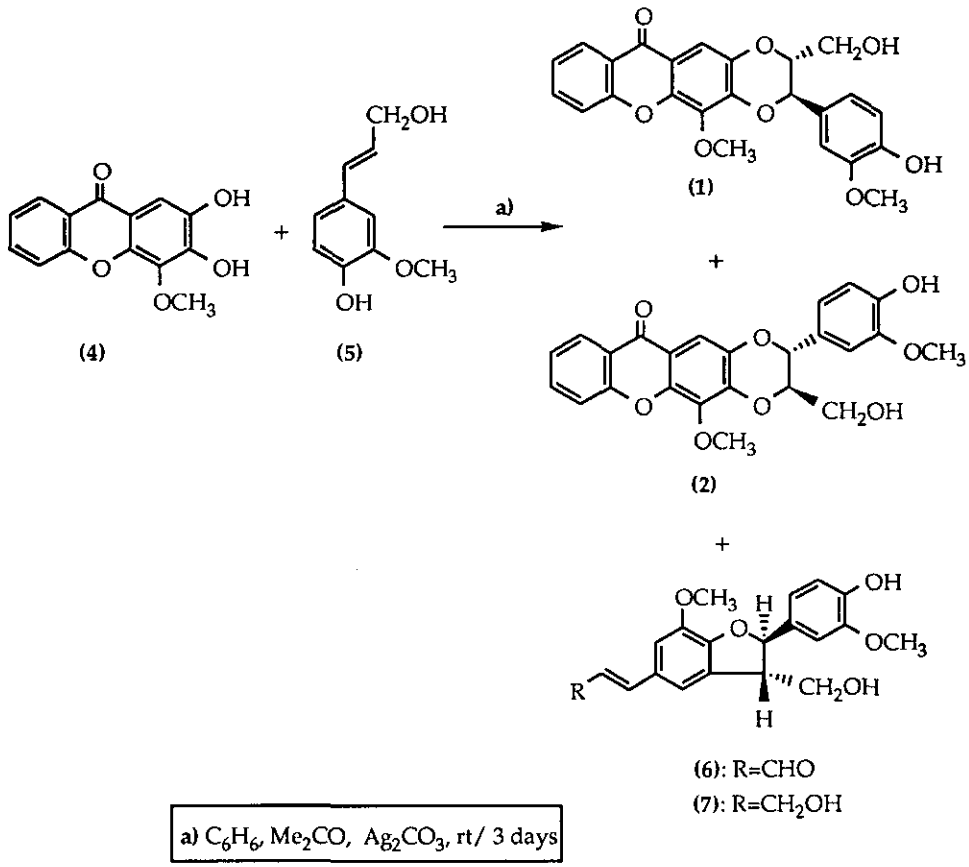
Figure 1

RESULTS AND DISCUSSION

The main step in the synthesis of *trans*-(±)-kielcorin B (**1**) was the oxidative coupling of 2,3-dihydroxy-4-methoxyxanthone (**4**) (obtained as before[7]) with coniferyl alcohol (**5**), in the presence of silver carbonate. This step gave two isomeric xanthonolignoids, *trans*-(±)-kielcorin B (**1**) and *trans*-(±)-isokielcorin B (**2**);[8] two benzofuran derivatives, phenylcoumaran (**6**) and dehydrodiconiferyl alcohol (**7**) were also found (Scheme 1).[9,10]

Following our work on the structural characterisation of xanthones,[7] COSY experiments were used to assign almost all the proton resonances in the ^{1}H NMR spectra of *trans*-(±)-

kielcorin B (1) and *trans*-(±)-isokielcorin B (2), especially those of the ABCD spin system corresponding to H-5′, H-6′, H-7′ and H-8′.



Scheme 1

The assignment of 4′-OCH_3 and 3″-OCH_3 proton resonances was carried out by using COSYLR experiments, which correlates the long-range coupled protons. In the case of compound (**1**)the proton resonance at δ 3.88 ppm correlates with that of H-2″ (δ 7.10 ppm), whereas that at δ 4.05 ppm does not show any correlation. This fact prompted us to assign the former to 3″-OCH_3 and the latter to 4′-OCH_3 protons resonances. Similarly, in compound (**2**)the resonance at δ 3.88 ppm (3″-OCH_3) correlates with that of H-2″ (δ 7.16 ppm), whereas that at δ 4.12 (4′-OCH_3) shows no cross peak.

Taking into consideration the coupling constant values $J_{H2\text{-}H3}$ = 8.3 and 8.0 Hz in the spectra of **1** and **2** , *trans* configuration for H-2 and H-3 could be proposed. This hypothesis was confirmed by some NOE experiments: upon irradiation of H-3 resonances of both compounds (δ 5.24 ppm for **1**, and δ 5.13 ppm for **2**), NOE effects were observed on the

resonances of H-2'' and H-6'', but no NOE effect was observed in that of H-3. These results are only compatible with the proposed *trans* configuration.

In the ^{13}C NMR spectra of compounds (**1**) and (**2**), the resonances of all protonated carbon atoms were assigned by using HETCOR experiments and comparing with data from previous studies on the structural characterization of xanthones.[7]

The determination of the substituents position on the 1,4-dioxane moiety, a persistent problem in the structure characterisation of xanthonolignoids,[11] was carried out by using one-dimensional selective INEPT experiments.[12]

In the case of *trans*-(±)-kielcorin B (**1**), on irradiation of the H-1′ resonance (δ 7.51 ppm), with a long-range J (C/H) coupling optimized to 7 Hz, enhancements on the carbon resonances at δ 144.1 and 144.7 ppm, and also on the signals of C-9′ (δ 175.0 ppm) and C-8′a (δ 120.5 ppm) were observed. The enhancement of the signal of C-8′a is due to the proximity of H-7′ (δ 7.57 ppm) and H-1′ signals. The irradiation of the H-3 resonance (δ 5.24 ppm), with a long-range J (C/H) coupling optimized to 1 Hz,[11] led to the enhancement of a carbon resonance at δ 144.1 ppm. All these results prompted us to assign the C-3′ and C-4′a carbon resonances at, respectively, δ 144.1 and 144.7 ppm, and also to establish the substituents position of the 1,4-dioxane moiety as shown in structure (**1**) (Figure 2).

In the case of compound (**2**), upon irradiation of the H-1′ proton resonance (δ 7.46 ppm), with a long-range J (C/H) coupling optimized to 7 Hz, enhancements were observed on the carbon resonances at δ 144.1 and 144.8 ppm and also on signals of C-9′ (δ 175.0 ppm), C-8′a (δ 120.5 ppm) and C-4′b (δ 155.6 ppm) carbon atoms. The appearance of the signals of C-4′b and C-8′a are due to the proximity between the H-7′ (δ 7.56 ppm) and H-1′ proton resonances. The irradiation of the H-3 resonance (δ 5.13 ppm), with a long-range J (C/H) coupling optimized to 1 Hz, led to the enhancement of carbon resonance at δ 141.6 ppm. All these results prompted us to assign the C-2′, C-3′ and C-4′a carbon resonances at, respectively, δ 141.6, 144.1 and 144.7 ppm, and also to establish the substituents position of the 1,4-dioxane moiety as shown in structure (**2**) (Figure 2).

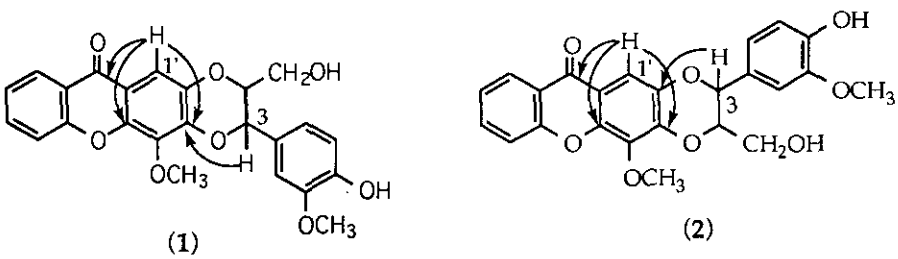


Figure 2

The assignments of the other quaternary carbons of compounds (1) and (2) were achieved by using one-dimensional selective INEPTexperiments, as shown in the Table 1.

The assignments of all carbon resonances of compounds (1) and (2) were confirmed by using HMBC experiments. In the case of compound (2), this experiment was also useful for the assignment of the C-8'a resonance: in this spectrum it was possible to observe correlation between the signals of H-6' and H-8' and the resonance at δ 120.5 ppm, which was then attributed to C-8'a.

Table 1. ^{1}H-^{13}C Long-Range Correlations of *trans*-(±)-Kielcorin B (1) and *trans*-(±)-Isokielcorin B (2) Determined by One-Dimensional Selective INEPT

Compound	Irradiated Proton Atoms (δ, ppm)	Resonances of Carbon Atoms (δ, ppm)
1	3''-OCH_3 (3.88)	147.8 (C-3'')
	4'-OCH_3 (4.05)	136.1 (C-4')
	H-3 (5.24)	77.8 (C-2), 111.7 (C-2''), 120.7 (C-6'') and 126.7 (C-1'')
	H-5' (7.80)	120.5 (C-8'a) and 155.6 (C-4'b)
	H-8' (8.27)	155.6 (C-4'b) and 175.0 (C-9')
	4''-OH (9.39)	147.3 (C-4'') and 147.8 (C-3'')
2	3''-OCH_3 (3.88)	147.7 (C-3'')
	4'-OCH_3 (4.12)	136.1 (C-4')
	H-3 (5.13)	111.7 (C-2''), 120.7 (C-6'') and 126.8 (C-1'')
	H-8' (8.26)	155.6 (C-4'b) and 175.0 (C-9')
	4''-OH (9.35)	115.4 (C-5''), 147.2 (C-4''), and 147.7 (C-3'')

From the comparison of the ^{1}H and ^{13}C NMR resonances of *trans*-(±)-kielcorin B (1) and *trans*-(±)-isokielcorin B (2) (Figure 3) shows the downfield shifts of H-3 and C-3 of 1 relative to H-3 and C-3 of 2. Comparable downfield shifts are also observed for H-2 and C-2 of 2 relative to those of 1.

In both cases, the net downfield shifts are clearly caused by a strong deshielding mesomeric effect exerted by the carbonyl group, through the oxygen substituent on C-3', rather than the effect of the methoxy group at C-4' on H-3 and C-3 of 1 and on H-2 and C-2 of the compound (2).

The MS spectra obtained for 1 and 2 showed the molecular ion at m/z 346 and a fragmentation characteristic of the xanthonolignoid skeleton.[5,13,14] Their MS spectra were similar to that of *trans*-(±)-kielcorin (3),[13] and showed a peak at m/z 180, corresponding to the retro-Diels-Alder cleavage of the 1,4-dioxane ring, and the peaks at m/z 162, 152, 151,

137, 124 indicating that the substituents of the phenylpropane unit are the same in both xanthonolignoids. The xanthone nucleus in **1** and **2** was evidenced from the ions at m/z 258, 256, 243, 228.[2, 5,13]

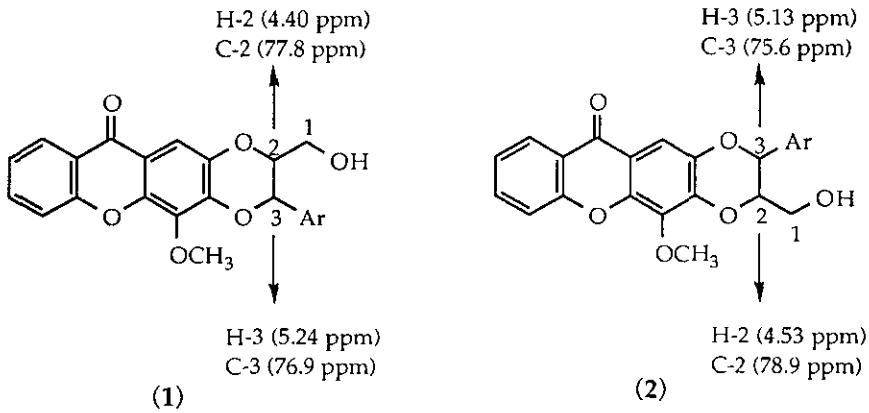


Figure 3

EXPERIMENTAL

Melting points were obtained in a Köfler microscope and are uncorrected. IR spectra were recorded on a Perkin Elmer 257 in KBr. ^{1}H and ^{13}C NMR spectra were taken in DMSO-d_6 at ambient temperature, on a Bruker AMX 300 instrument operating at 300.13 and 75.47 MHz, respectively. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference. In a typical measurement of ^{1}H NMR spectrum, the spectral width was 5.55 KHz, the aquisition time 2.95 s and the pulse width of 4.13 μs (33.3°). In the broadband decoupling ^{13}C NMR spectra the spectral width was 18.5 KHz, the aquisition time 0.885 s and the pulse width of 1.5 μs (33.3°). COSY, HETCOR, HMBC and one-dimensional selective INEPT[11] experiments were carried out using Bruker microprograms. In the case of HMBC, the low-pass *J*-filter portion of the experiment was optimized for an average of one-bond heteronuclear coupling of 145 Hz; the delay for evolution of long-range couplings was optimized for 10 Hz. NOE experiments were determined by means of NOE difference technique, using an irradiation time of 2 s and a relaxation delay of 4 s.

MS spectra were recorded as EI (electronic impact) mode on a Hitachi Perkin-Elmer, RMU-6M spectrometer. HRMS spectra were recorded as EI (electronic impact) mode on AutoSpec-QVG spectrometer. Purifications of compounds were performed by column chromatography, using Merck silica gel 60 (0.50-0.20 mm), and preparative TLC, employing Merck silica gel 60 (GF_{254}).

Synthesis of *trans*-(±)-kielcorin B (1) and *trans*-(±)-isokielcorin B (2)

A mixture of 2,3-dihydroxy-4-methoxyxanthone (4) (700.3 mg, 2.7 mmol) and coniferyl alcohol (**5**) (565.8 mg, 3.1 mmol) was taken in a mixture of 1:1 benzene:acetone (50 mL) and stirred in the presence of silver carbonate (1.1 g, 3.8 mol), in the dark, for 3 days. The suspension was filtered, the filtrate evaporated, and the crude product purified by column chromatography using a mixture of 3:7 hexane:chloroform as eluent. The isolation of *trans*-(±)-kielcorin B (**1**) (10 mg), *trans*-(±)-isokielcorin B (**2**) (20 mg), phenylcoumaran (**6**) (18 mg) and dehydrodiconiferyl alcohol (7) (35 mg) was then carried out by preparative TLC using a mixture of 30:1 $CHCl_3$:MeOH.

trans-(±)-kielcorin B (**1**); mp: 232-236°C; IR ν_{max} (cm^{-1}) 3427, 1653, 1606, 1516, 1456, 1381, 1329, 1265, 1217, 1128, 1095, 1030, 804; ^{1}H NMR δ 3.57 (2H, m, C*H*$_2$OH), 3.88 (3H, s, 3″-OCH3), 4.05 (3H, s, 4′-OC*H*$_3$), 4.40 (1H, m, H-2), 5.24 (1H, d, J=8.3 Hz, H-3), 6.93 (1H, d, J=8.1 Hz, H-5″), 7.03 (1H, d, J=8.1 Hz, H-6″), 7.19 (1H, s, H-2″), 7.51 (1H, s, H-1′), 7.57 (1H, dd, J=7.7 Hz, J=7.4 Hz, H-7′), 7.80 (1H, d, J=8.1 Hz, H-5′), 7.95 (1H, ddd, J=8.1 Hz, J=7.4 Hz, J=1.5 Hz, H-6′), 8.27 (1H, d, J=7.7 Hz, H-8′), 9.39 (1H, s, 4″-O*H*); ^{13}C NMR δ 55.7 (3″-OCH_3), 60.0 (CH_2OH), 61.3 (4′-OCH_3), 76.9 (C-3), 77.8 (C-2), 105.4 (C-1′), 111.7 (C-2″), 114.8 (C-8′b), 115.4 (C-5″), 118.3 (C-5′), 120.5 (C-8′a), 120.7 (C-6″), 124.3 (C-7′), 125.9 (C-8′), 126.7 (C-1″), 135.1 (C-6′), 136.1 (C-4′), 141.6 (C-2′), 144.1 (C-3′), 144.7 (C-4′a), 147.3 (C-4″), 147.8 (C-3″), 155.6 (C-4′b), 175.0 (C-9′); MS m/z (rel int) 346 (100, M^+), 418 (78), 419 (22), 406 (14), 405 (8), 404 (7), 403 (12), 300 (18), 299 (83), 283 (12), 269 (36), 258 (24), 256 (3), 243 (12), 228 (9), 215 (6), 187 (5), 181 (11), 180 (65), 179 (7), 178 (5), 163 (8), 162 (40), 161 (20), 157 (21), 152 (7), 151 (10), 150 (16), 149 (10), 147 (17), 142 (40), 138 (10), 137 (74), 131 (11), 127 (12), 124 (33), 119 (21), 101 (12), 97 (18), 91 (27), 84 (19), 83 (25), 81 (29), 76 (17), 71 (29), 69 (70), 58 (27), 57 (64). HRMS Calcd for $C_{24}H_{20}O_8$: 436.1158. Found 436.1161.

trans-(±)-Isokielcorin B (**2**); mp: 108-110°C; IR ν_{max} (cm^{-1}) 3432, 1640, 1610, 1516, 1460, 1382, 1332, 1263, 1101, 1032, 803; ^{1}H NMR δ 3.78 (2H, m, C*H*$_2$OH), 3.88 (3H, s, 3″-OC*H*$_3$), 4.12 (3H, s, 4′-OC*H*$_3$), 4.53 (1H, m, H-2), 5.13 (1H, d, J=8.0 Hz, H-3), 6.92 (1H, d, J=7.7 Hz, H-5″), 7.00 (1H, dd, J=7.7 Hz, J=1.6 Hz, H-6″), 7.16 (1H, d, J=1.6 Hz, H-2″), 7.46 (1H, s, H-1′), 7.56 (1H, dd, J=7.6Hz, J=7.2 Hz, H-7′), 7.80 (1H, d, J=8.4 Hz, H-5′), 7.95 (1H, dt, J=7.6 Hz, J=1.5 Hz, H-6′), 8.26 (1H, dd, J=7.2 Hz, J=1.5 Hz, H-8′), 9.35 (1H, s, 4″-O*H*); ^{13}C NMR δ 55.7 (3″-OCH_3), 59.9 (CH_2OH), 61.3 (4′-OCH_3), 75.6 (C-3), 78.9 (C-2), 105.5 (C-1′), 111.7 (C-2″), 114.8 (C-8′b), 115.4 (C-5″), 118.3 (C-5′), 120.5 (C-8′a), 120.7 (C-6″), 124.3 (C-7′), 125.9 (C-8′), 126.8 (C-1″), 135.1 (C-6′), 136.1 (C-4′), 141.6 (C-2′), 144.1 (C-3′), 144.8 (C-4′a), 147.2 (C-4″), 147.7 (C-3″), 155.6 (C-4′b), 175.0 (C-9′); MS m/z (rel int) 436 (89, M^+), 418 (43), 419 (12), 406 (8), 405 (6), 404 (3), 403 (6), 300 (5), 299 (24), 270 (5), 269 (23), 258 (10), 257 (5), 256 (3), 255 (9), 254 (5), 243 (5), 228 (8), 225 (5), 215 (2), 187 (2), 181 (19), 180 (100), 178 (4), 179 (9), 163 (6), 162 (28), 161 (13), 157 (18), 152 (11), 151 (12), 150 (14), 149 (10), 147 (10), 138 (12), 137 (82), 135 (5), 131 (9), 125 (6), 124 (46), 119 (14), 91 (20), 77 (14), 65 (10), 55 (13). HRMS Calcd for $C_{24}H_{20}O_8$: 436.1158. Found 436.1153.

Phenylcoumaran (6) and dehydrodiconiferyl alcohol (7) - data according to literature.[13]

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