Lignans and Triterpenoids from the Stems of *Kadsura induta*

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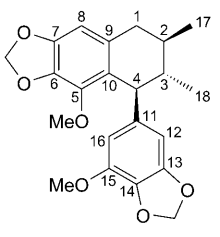
Two new lignans, kadsurindutins C and H (**1** and **2**, resp.), and a new 18(13→12)-*abeo*-lanostane triterpenoid acid, kadindutic acid (**3**), were isolated from the stems of *Kadsura induta*, together with known lignans and triterpenoids. Their structures and configurations were elucidated by spectroscopic methods, including 2D-NMR techniques.

Introduction. – Plants of the family Schisandraceae have proved to be a rich source of dibenzocyclooctane lignans, as well as lanostane and cycloartane triterpenes, which have been found to possess many beneficial pharmacological effects, such as anti-lipid peroxidative, antitumor, anti-HIV, and anti-HBV activities [1–5]. *Kadsura induta* is a medicinal plant indigenous to southern China. Its stems are commonly used for the treatment of painful aching of joints and blood deficiency in Chinese folk medicine. In our previous study, two new dibenzocyclooctane lignans, kadsurindutins A and B, were isolated from *K. induta*, and some lignans were found to show antiviral effect on hepatitis B virus [6]. Further phytochemical investigation on the stems of *K. induta* led to the isolation of a new 4-aryltetralin lignan, kadsurindutin C (**1**), a new dibenzocyclooctane lignan, kadsurindutin H (**2**), and a new 18(13→12)-*abeo*-lanostane triterpenoid acid, named kadindutic acid (**3**), as well as four known compounds, kadsuphilin B, anwuweizic acid, epianwuweizic acid, and (24*Z*)-12α-hydroxy-3-oxolanosta-8,24-dien-26-oic acid. In this report, we describe the isolation and characterization of the new compounds **1**–**3**.



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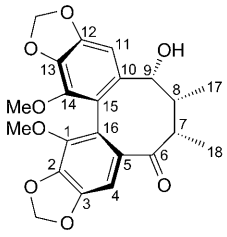

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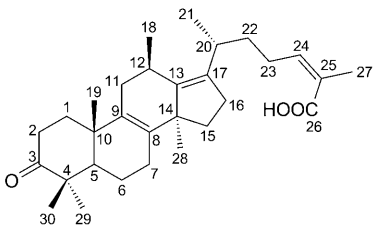

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[1]) These two authors have contributed equally to this work.

[2]) Arbitrary numbering. For systematic names, see *Exper. Part.*

Results and Discussion. – Repeated column chromatography of the Et_2O extract from the stems of *K. induta* yielded seven compounds.

Kadsurindutin C (**1**) was obtained as a yellow powder and shown to possess the molecular formula $C_{22}H_{24}O$ by HR-ESI-MS (*m/z* 385.1654 ($[M+H]^+$)) with eleven degrees of unsaturation. Detailed analysis of the NMR and DEPT spectra (*Table 1*) showed that **1** contains two MeO, two Me, three CH_2, and six CH groups, as well as nine quaternary C-atoms. The UV spectrum of **1**, with maxima at 211 and 282 nm, along with the corresponding NMR spectra suggested that **1** was a 4-aryltetralin type lignan.

The ^{1}H-NMR spectrum of **1** accounted for the presence of three aromatic H-atoms at δ(H) 6.11, 6.23, and 6.36, respectively. The aromatic H-atom signal at δ(H) 6.36 (*s*, 1 H), which displayed HMBC with C(1)[2]) at δ(C) 33.5 and C(9) at δ(C) 122.2, as well as ROESY cross-peaks (*Fig. 1*) with CH_2(1) at δ(H) 2.35 and 2.65 (*dd*, J = 5.5, 17.2, 1 H each), was assigned to H–C(8). The aromatic H-atom signal at δ(H) 6.23 (*s*, 1 H), which showed a HMBC cross-peak with C(4) at δ(C) 47.2 and a ROESY cross-peak with MeO–C(15) at δ(H) 3.86 (*s*, 3 H), was assigned to H–C(16). The third aromatic H-atom signal at δ(H) 6.11 was due to H–C(12) on the basis of a HMBC with C(4). The *doublet*s at δ(H) 0.87 (*d*, J = 7.0, Me(17)) and 0.90 (*d*, J = 7.0, Me(18)) suggested C(2) and C(3) to be of the CH type. The signals at δ(H) 5.86 and 5.87 (*s*, 1 H each), and 5.90 (*s*, 2 H), and at δ(C) 100.6 and 101.0 indicated the presence of two OCH_2O groups, which were determined to be located at C(6,7) and C(13,14), respectively, on the basis of HMBC of H–C(8) with C(6) and C(7), and of H–C(12) with C(13) and C(14), as well as HMBC between the corresponding OCH_2O resonances with C(6) and C(7),

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**[2]) (at 400 and 100 MHz, resp., in $CDCl_3$ at 27°; δ in ppm, J in Hz)

Position	δ(H)	δ(C)
1	2.35 (*dd*, J = 5.5, 17.2, 1 H), 2.65 (*dd*, J = 5.5, 17.2, 1 H)	33.5 (*t*)
2	1.94–2.02 (*m*, 1 H)	25.7 (*d*)
3	1.82–1.86 (*m*, 1 H)	40.7 (*d*)
4	4.00 (*d*, J = 2.4, 1 H)	47.2 (*d*)
5	–	141.1 (*s*)
6	–	134.9 (*s*)
7	–	147.7 (*s*)
8	6.36 (*s*, 1 H)	102.6 (*d*)
9	–	122.2 (*s*)
10	–	130.9 (*s*)
11	–	142.9 (*s*)
12	6.11 (*s*, 1 H)	102.5 (*d*)
13	–	148.1 (*s*)
14	–	132.8 (*s*)
15	–	142.9 (*s*)
16	6.23 (*s*, 1 H)	107.7 (*d*)
17	0.87 (*d*, J = 7.0, 3 H)	18.8 (*q*)
18	0.90 (*d*, J = 7.0, 3 H)	13.6 (*q*)
*Me*O–C(5)	3.60 (*s*, 3 H)	59.3 (*q*)
*Me*O–C(15)	3.86 (*s*, 3 H)	56.6 (*q*)
C(6)–(O*CH$_2$*O)–C(7)	5.86 (*s*, 1 H), 5.87 (*s*, 1 H)	100.6 (*t*)
C(13)–(O*CH$_2$*O)–C(14)	5.90 (*s*, 2 H)	101.0 (*t*)

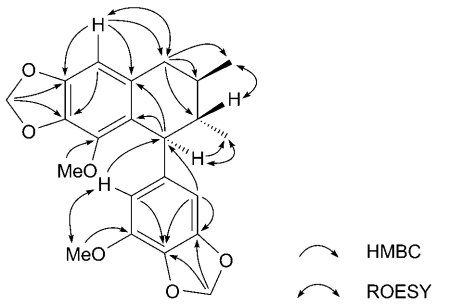

Fig. 1. *Key HMBC and ROESY correlations in* **1**

and with C(13) and C(14), respectively. The MeO at δ(H) 3.60 (*s*, 3 H) is connected to C(5), because of a HMBC correlation with C(5) at δ(C) 141.1.

The ROESY cross-peaks (*Fig. 1*) between H–C(1) and Me(17), H–C(3) and Me(17), as well as H–C(4) and Me(18) indicated that the aryl and the two Me groups are in a *trans*–*trans* relationship to each other [7]. In addition, a positive CD *Cotton* effect at 289 nm let us conclude that **1** is a (2*R*,3*S*,4*S*)-aryl-tetralin[2]) derivative [8].

Finally, the structure of **1** was elucidated as (5*S*,6*S*,7*R*)-5,6,7,8-tetrahydro-4-methoxy-5-(7-methoxy-1,3-benzodioxol-5-yl)-6,7-dimethylnaphtho[2,3-*d*][1,3]dioxole.

Kadsurindutin H (**2**) was obtained as a yellow powder and had the molecular formula $C_{22}H_{22}O_8$, as determined by HR-ESI-MS (*m*/*z* 437.1207 ([M + Na]$^+$)). The UV spectrum with a maximum absorption at 212 nm and two shoulders at 235 and 282 nm, along with the corresponding NMR spectra (*Table 2*) indicated that **2** was a dibenzocyclooctane-type lignan [9].

The resonances at δ(H) 0.90 (*d*, J = 7.4, 3 H) and 1.01 (*d*, J = 6.7, 3 H) could be assigned to the *cis*-oriented Me(17) and Me(18)[2]) [10], respectively. An additional absorption at 323 nm (log ε 3.25) in the UV spectrum of **2**, along with IR absorption at 1653 cm^{-1} and signal at δ(C) 200.5 in the ^{13}C-NMR spectrum, revealed the presence of a conjugated ketone moiety [11]. The signal at δ(H) 7.45 (*s*, H–C(4), 1 H), shifted downfield by *ca.* 1 ppm as compared with 'common' aromatic H-atoms, which showed HMBC (*Fig.* 2) with the C=O group at δ(C) 200.5, indicated that the CO group was located at C(6) and conjugated with the aromatic ring [12][13]. The ^{1}H-NMR spectrum also showed signals for two MeO groups at δ(H) 3.78 and 3.87 (*s*), two OCH_2O moieties at δ(H) 6.05, 6.06 (*d*, J = 1.2, 1 H each), and 6.02, 6.04 (*d*, J = 1.4, 1 H each) at two aromatic rings. The two OCH_2O moieties were attached to C(12) (δ(C) 149.5) and C(13) (δ(C) 136.1), and C(2) (δ(C) 141.1) and C(3) (δ(C) 149.2), respectively, on the basis of HMBC of H–C(11) with C(12) and C(13), and of H–C(4) with C(2) and C(3), as well as HMBC between the corresponding OCH_2O resonances with C(12) and C(13), and with C(2) and C(3), respectively. The IR absorption at 3490 cm^{-1} suggested the presence of an OH group, the NMR signals at δ(H) 4.77 (H–C(9)) and δ(C) 79.0 (C(9)) indicated an OH group at C(9), similar as kadsurindutin B [6], which was further supported by the HMBC correlation between H–C(11) and C(9). A ROESY cross-peak (*Fig. 2*) between H–C(11) and H–C(9) indicated that the HO–C(9) group was α-oriented.

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2**[2]) (at 400 and 100 MHz, resp., in $CDCl_3$ at 27°; δ in ppm, J in Hz)

Position	δ(H)	δ(C)
1	–	140.9 (*s*)
2	–	141.1 (*s*)
3	–	149.2 (*s*)
4	7.45 (*s*, 1 H)	104.2 (*d*)
5	–	131.1 (*s*)
6	–	200.5 (*s*)
7	2.79–2.82 (*m*, 1 H)	44.5 (*d*)
8	1.95–1.99 (*m*, 1 H)	47.4 (*d*)
9	4.77 (*s*, 1 H)	79.0 (*d*)
10	–	135.9 (*s*)
11	6.37 (*s*, 1 H)	101.3 (*d*)
12	–	149.5 (*s*)
13	–	136.1 (*s*)
14	–	142.2 (*s*)
15	–	119.1 (*s*)
16	–	125.7 (*s*)
17	0.90 (*d*, J = 7.4, 3 H)	10.0 (*q*)
18	1.01 (*d*, J = 6.7, 3 H)	15.8 (*q*)
*Me*O–C(1)	3.78 (*s*, 3 H)	59.8 (*q*)
*Me*O–C(14)	3.87 (*s*, 3 H)	59.8 (*q*)
C(2)–(O*CH*$_2$O)–C(3)	6.06, 6.05 (*d*, J = 1.2, each 1 H)	101.9 (*t*)
C(12)–(O*CH*$_2$O)–C(13)	6.04, 6.02 (*d*, J = 1.4, each 1 H)	101.4 (*t*)
HO–C(9)	1.39 (br. *s*, 1 H)	

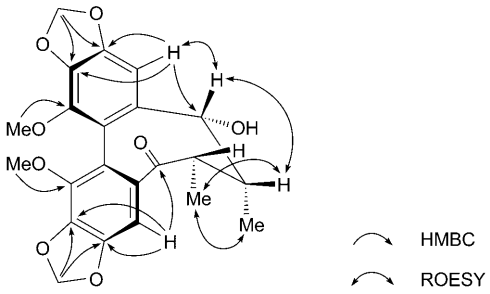

Fig. 2. *Key HMBC and ROESY correlations in* **2**

The circular dichroism (CD) spectrum showed negative and positive *Cotton* effects at 340 and 280 nm, respectively, indicating that **2** contains an axially chiral (a*S*)-1,1′-biphenyl unit ((*P*)-helicity) [14]. Since the aromatic ring was conjugated with the ketone group, a twist-boat (TB) conformation is the only possible conformation for the dibenzocyclooctane ring system [15]. The result was corroborated by a ROESY experiment, exhibiting correlations of H–C(11) with H–C(9), Me(18) with H–C(8), H–C(8) with H–C(11), and Me(17) with Me(18). The structure of **2** was thus determined as (6*S*,7*R*,8*R*)-7,8-dihydro-8-hydroxy-13,14-dimethoxy-6,7-dimethyl-1,3-benzodioxolo[5′,6′:3,4]cycloocta[1,2-*f*][1,3]benzodioxol-5(6*H*)-one.

Kadindutic acid (**3**), obtained as a white powder, had the molecular formula $C_{30}H_{46}O_3$, according to the HR-ESI-MS (*m/z* 454.3441 (M^+)). The IR spectrum displayed absorptions for a conjugated acid (1694, 2700–3100 cm^{-1}) and a ketone (1703 cm^{-1}). The ^{1}H-NMR spectrum (*Table 3*) showed an angelic acid unit (δ(H) 6.10, $t, J = 7.4$, H–C(24); δ(H) 1.92, *s*, Me(27)), two secondary Me groups (δ(H) 1.12, $d, J = 6.8$; δ(H) 0.95, $d, J = 6.3$), four tertiary Me groups (δ(H) 0.97, 0.94, 1.05, and 1.10, each *s*). These features resembled those of anwuweizonic acid (**5**) [16], suggesting that **3** was also a lanostane triterpenoid acid.

Table 3. *^{1}H- and ^{13}C-NMR Data of* **3** (at 400 and 100 MHz, resp., in $CDCl_3$ at 27°; δ in ppm, *J* in Hz)

Position	δ(H)	δ(C)
1	1.96–2.00 (*m*, 1 H), 1.67–1.71 (*m*, 1 H)	35.8 (*t*)
2	2.48–2.52 (*m*, 2 H)	34.3 (*t*)
3	–	218.7 (*s*)
4	–	47.0 (*s*)
5	1.70–1.76 (*m*, 1 H)	51.0 (*d*)
6	1.60–1.64 (*m*, 1 H), 1.46–1.50 (*m*, 1 H)	20.0 (*t*)
7	2.00–2.06 (*m*, 1 H), 2.08–2.14 (*m*, 1 H)	26.1 (*t*)
8	–	135.7 (*s*)
9	–	134.2 (*s*)
10	–	37.0 (*s*)
11	1.72–1.76 (*m*, 1 H), 2.16–2.20 (*m*, 1 H)	33.3 (*t*)
12	2.50–2.54 (*m*, 1 H)	29.3 (*d*)
13	–	143.8 (*s*)
14	–	51.7 (*s*)
15	1.43–1.47 (*m*, 1 H), 1.65–1.69 (*m*, 1 H)	37.3 (*t*)
16	2.03–2.09 (*m*, 1 H), 2.19–2.25 (*m*, 1 H)	27.6 (*t*)
17	–	134.6 (*s*)
18	1.12 ($d, J = 6.8$, 3 H)	21.5 (*q*)
19	0.97 (*s*, 3 H)	19.8 (*q*)
20	2.72–2.76 (*m*, 1 H)	32.0 (*d*)
21	0.95 ($d, J = 6.3$, 3 H)	19.7 (*q*)
22	1.42–1.45 (*m*, 1 H), 1.59–1.63 (*m*, 1 H)	35.1 (*t*)
23	2.40–2.44 (*m*, 2 H)	28.5 (*t*)
24	6.10 ($t, J = 7.4$, 1 H)	146.9 (*q*)
25	–	125.6 (*s*)
26	–	171.6 (*s*)
27	1.92 (*s*, 3 H)	20.5 (*q*)
28	0.94 (*s*, 3 H)	22.3 (*q*)
29	1.05 (*s*, 3 H)	20.7 (*q*)
30	1.10 (*s*, 3 H)	27.2 (*q*)

In the HMBC spectrum of **3** (*Fig. 3*), the H-atom signals at δ(H) 1.96–2.00 and 1.67–1.71 (H–C(1)), 2.48–2.52 (H–C(2)), 1.05 (Me(29)), and 1.10 (Me(30)) were correlated with the C-atom signal at δ(C) 218.7, suggesting that the ketone group was positioned at C(3). HMBC of Me(19) at δ(H) 0.97 with C(9) at δ(C) 134.2, H–C(7) at δ(H) 2.08–2.14 and 2.00–2.06, H–C(11) at δ(H) 1.72–1.76 and 2.16–2.20 with C(8) at δ(C) 135.7 and C(9) suggested a C(8)=C(9) bond, as in anwuweizonic acid. Comparison of the ^{1}H-NMR spectra of **3** and anwuweizonic acid showed that the C(13)

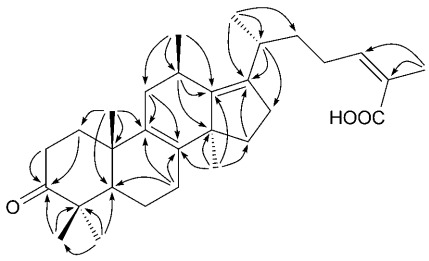

Fig. 3. *Key HMBC in* **3**

tertiary Me signal present in the latter at δ(H) 0.71 (Me(18)) had disappeared in **3**, instead appearing as a secondary Me signal at δ(H) 1.12 (d, $J = 6.8$), leading to the formation of a tetra-substituted C(13)=C(17) bond in **3**, as that of ananosic acid A [17]. HMBC between the signals at δ(H) 1.12 (d, $J = 6.8$, 3 H) with C(11) at δ(C) 33.3, C(12) at δ(C) 29.3, and C(13) at δ(C) 143.8, indicated that the Me group was attached to C(12).

Finally, **3** was elucidated as (2*Z*,6*R*)-2-methyl-6-[(12β)-4,4,10,12,14-pentamethyl-3-oxogona-8,13(17)-dien-17-yl]hept-2-enoic acid.

The known compounds were identified as kadsuphilin B [18], anwuweizonic acid [16], epianwuweizic acid [19] and (24*Z*)-12α-hydroxy-3-oxolanosta-8,24-dien-26-oic acid [20] by comparison of the CD, UV, IR, and NMR data with those reported. All the known compounds were isolated from *K. induta* for the first time. Anwuweizonic acid and (24*Z*)-12α-hydroxy-3-oxolanosta-8,24-dien-26-oic acid were reported to show strong cholesterol biosynthesis inhibitory activity [21].

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Experimental Part

General. Anal. TLC: silica-gel plates (SiO_2; *Yan-tai Institute of Chemical Technology*), with petroleum ether (PE)/acetone 3 : 1 as eluent; visualization under UV light, and by spraying with 10% aq. H_2SO_4, followed by heating. Column chromatography (CC): silica gel (SiO_2; 200 – 300, or 300 – 400 mesh; *Qingdao Marine Chemical Factory*). Optical rotations (ORD): *JASCO P-1020* spectropolarimeter. UV Spectra: *Shimadzu UV-260* spectrophotometer, in anh. MeOH; λ_{max} (log ε) in nm. CD Spectra: *JASCO J-715* spectropolarimeter; λ in nm ($\Delta\varepsilon$). IR Spectra: *Avatar 360-ESP* spectrophotometer (*Thermo Nicolet*), as KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *DRX-400* spectrometer, in $CDCl_3$; δ in ppm, *J* in Hz. EI-MS: *HP-5989A* mass spectrometer; in *m*/*z* (rel. %). HR-ESI-MS: *Bruker APEX 7.0 TESLA FT-MS* apparatus.

Plant Material. The stems of *Kadsura induta* were collected in Pingbian County,Yunnan Province, P. R. China, in July of 2004. A voucher specimen (DFC-MA200401) is deposited with the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried ground stems (10 kg) of *K. induta* were extracted exhaustively with 95% aq. EtOH at r.t. The EtOH extract was concentrated *in vacuo* to yield a semi-solid (1.57 kg), which was suspended in H_2O (1000 ml), and extracted with Et_2O (7 × 350 ml). The combined org. phase was concentrated to yield a residue (400 g), part of which (200 g) was subjected to CC (2 kg SiO_2; PE/

acetone gradient) to afford nine fractions (*Fr. 1–Fr. 9*). *Fr. 3*, eluted with PE/acetone 30:1, was subjected to repeated CC (SiO_2; PE/acetone 40:1) to afford **1** (2 mg). *Fr. 4*, eluted with PE/acetone 20:1, was subjected to repeated CC (SiO_2; with PE/acetone 30:1) to yield **3** (1 mg). *Fr. 5*, eluted with PE/acetone 10:1, was subjected to repeated CC (SiO_2; with PE/acetone 15:1) to afford anwuweizonic acid (5 mg) and epianwuweizic acid (4 mg). *Fr. 8*, eluted with PE/acetone 3:1, was subjected to repeated CC (SiO_2; with PE/acetone 4:1), and then with prep. TLC (PE/acetone 2:1) to yield **2** (3 mg) and kadsuphilin B (2 mg). *Fr. 9*, eluted with PE/acetone 3:2, was subjected to repeated CC (SiO_2; with $CHCl_3$/MeOH 30:1) to afford (24*Z*)-12α-hydroxy-3-oxolanosta-8,24-dien-26-oic acid (2 mg).

Kadsurindutin C (=*(5*S*,6*S*,7*R*)-5,6,7,8-Tetrahydro-4-methoxy-5-(7-methoxy-1,3-benzodioxol-5-yl)-6,7-dimethylnaphtho[2,3-*d*][1,3]dioxole*; **1**). Yellow powder. $[\alpha]_D^{25} = -36.0$ ($c = 0.01$, MeOH). UV (MeOH): 211 (4.66), 282 (sh, 3.56). CD ($c = 0.01$, MeOH): 240 (+59), 275 (−28), 289 (+14). IR (KBr): 3443, 2918, 1631, 1475, 1088, 1048, 732. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 384 (17, M^+), 327 (11), 297 (9), 232 (10), 178 (19), 165 (12), 97 (34), 85 (32), 69 (53), 57 (100), 43 (75), 41 (64). HR-ESI-MS: 385.1654 ($[M+H]^+$, $C_{22}H_{25}O_6^+$; calc. 385.1651).

Kadsurindutin H (=*(6*S*,7*R*,8*R*)-7,8-Dihydro-8-hydroxy-13,14-dimethoxy-6,7-dimethyl-1,3-benzodioxolo[5′,6′:3,4]cycloocta[1,2-*f*][1,3]benzodioxol-5(6*H*)-one*; **2**). Yellow powder. $[\alpha]_D^{25} = +12.1$ ($c =$ 0.1, MeOH). UV (MeOH): 212 (2.38), 235 (sh, 1.81), 282 (sh, 0.64), 323 (3.25). CD ($c = 0.1$, MeOH): 280 (+5), 340 (−28). IR (KBr): 3490, 2935, 2360, 1653, 1615, 1083, 975, 731. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 415.1 ($[M+H]^+$). HR-ESI-MS: 437.1207 ($[M+Na]^+$, $C_{22}H_{22}NaO_8^+$; calc. 437.1210).

Kadindutic Acid (=*(2*Z*,6*R*)-2-Methyl-6-[(12β)-4,4,10,12,14-pentamethyl-3-oxogona-8,13(17)-dien-17-yl]hept-2-enoic Acid*; **3**). White powder. $[\alpha]_D^{25} = +56.0$ ($c = 0.005$, MeOH). IR (KBr): 3416, 2700–3100, 1958, 1703, 1694, 1459, 1383, 1047, 914. ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 454.3441 (M^+, $C_{30}H_{46}O_3^+$; calc. 454.3447).

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