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LIMONOIDS FROM *EKEBERGIA CAPENSIS*

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Key Word Index—*Ekebergia capensis*; Meliaceae; limonoid; methyl angolensate; capensolactones 1–3.

Abstract—The hexane extract of the seed of *Ekebergia capensis* (Meliaceae) yielded the novel limonoids, capensolactones 1–3 and the previously isolated methyl 3α-hydroxy-3-deoxyangolensate. Structures were determined by 2D NMR, mass spectroscopy and chemical methods.

INTRODUCTION

In continuation of our studies on members of the Southern African Meliaceae, the hexane extract of the seed of *Ekebergia capensis* Sparrm., collected in the Eastern Transvaal was examined and yielded the novel compounds, capensolactones 1–3 and the previously isolated methyl 3α-hydroxy-3-deoxyangolensate. The methanol extract yielded sucrose. Structures were determined by 2D NMR, mass spectroscopy and chemical means.

Ekebergia is a small genus of African trees belonging to the tribe Trichiliae of the Meliaceae. *E. capensis*, or the Cape Ash as it is known locally, is used medicinally and magically by the Zulu people. An extract from the bark is used to treat coughs and root extracts are used in the treatment of dysentery [1].

A previous investigation of the seed of this species collected in the Eastern Cape yielded ekebergin (**4**)[2]. An investigation into the seed of *Ekebergia senegalensis*, which is now considered conspecific with *E. capensis* [3], yielded the ekebergolactones A and B, similar in structure to capensolactones 1, 2a and 2b [4]. *Ekebergia pterophylla*, a related species, yielded ekebergin, (**4**)[5] related ekebergin-like compounds and methyl 3α-hydroxy-3-deoxyangolensate, **5**, which we also isolated in this work from *E. capensis*, and compounds whose structures were shown to be similar to those of the trijugins isolated from *Heynea trijuga* [6, 7].

RESULTS AND DISCUSSION

The air-dried peeled seed of *Ekebergia capensis* was extracted with hexane in a Soxhlet apparatus. The extract was partitioned between hexane and aqueous methanol to remove oils and the aqueous methanol layer was re-extracted with chloroform. The chloroform was removed and the resulting gum separated by repeated column chromatography over silica gel. This procedure yielded methyl 3α-hydroxy-3-deoxyangolensate and the capensolactones 1–3.

The structure of the first compound was identified as methyl 3α-hydroxy-3-deoxyangolensate, (**5**) by comparison of its NMR and mass spectroscopic data with literature values [8].

High resolution mass spectroscopy of capensolactone 1 showed that the compound had $[M]^+$ at *m/z* 678.2553 indicating a molecular formula of $C_{33}H_{42}O_{15}$. The loss of formaldehyde was indicated by a peak at *m/z* 648.2436 corresponding to a molecular formula $C_{32}H_{40}O_{14}$ $[M-30]^+$. A peak at *m/z* 588 corresponding to $[M-30-60]^+$ indicated the loss of acetic acid, suggesting the compound was a monoacetate. A peak at *m/z* 590 $[M-88]^+$ suggested the loss of a 2-methylpropionate ester. A sharp singlet at δ 2.14 (3H) confirmed that the compound was a monoacetate. A septet at δ 2.83 (J = 7.1 Hz) coupled to a six-proton doublet at δ 1.24 confirmed the presence of the 2-methylpropionate ester. The ^{1}H NMR spectrum of capensolactone 1 had the usual β-substituted furan ring resonances at δ 7.54 (H-21), 7.40 (H-23) and 6.57 (H-22). The HETCOR spectrum indicated that the corresponding carbon resonances occurred at δ 140.3 *d*, 143.1 *d*, and 109.1 *d*. The C-20 singlet occurred at δ 120.7. The ^{13}C NMR spectrum of capensolactone 1 also indicated the presence of a keto group (δ 208.4) and four ester carbonyl carbons (δ 176.7, 173.7, 168.3, and 166.4). There were three fully substituted C—O carbon atoms (δ 84.7, 83.5 and 59.2), six HC—O methine carbon resonances (δ 82.0, 76.0, 75.7, 74.5, 68.8, 67.4), a CH_3—O carbon resonance (δ 52.3) and a deshielded methylene carbon resonance which

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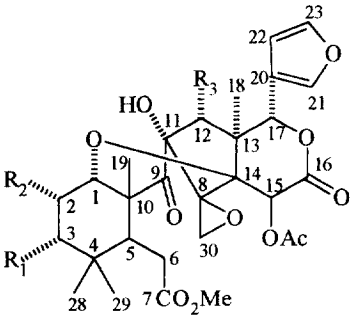
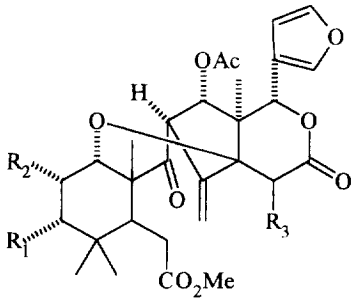
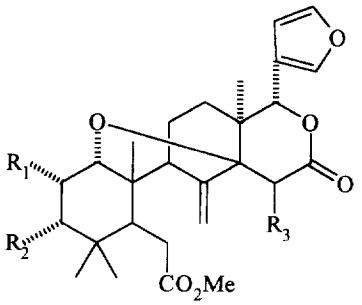


	R_1	R_2	R_3
1	OH	$(CH_3)_2CHCO_2$	OH
2a	ONicotinyl	$CH_3CH_2CH(CH_3)CO_2$	OAc
2b	$CH_3CH_2CH(CH_3)CO_2$	ONicotinyl	OAc



	R_1	R_2	R_3
3a	ONicotinyl	$(CH_3)_2CHCO_2$	OAc
3b	$(CH_3)_2CHCO_2$	ONicotinyl	OAc



	R_1	R_2	R_3
4	OH	$(CH_3)_2CHCO_2$	OAc
5	H	OH	H

occurred at δ 45.7. There were also three fully substituted carbon atoms, two methine, one methylene and seven methyl carbon resonances in the alkyl region of the spectrum.

The presence of a sharp singlet at δ 3.64(3H) indicated the presence of a carbomethoxy group at C-7, suggesting that ring B was opened. Usually limonoids with an opened ring B have a 8,30-double bond indicated by two H-30 resonances at δ 5.10 and δ 4.90 in compounds such as **4**. These resonances were not present in the ^{1}H NMR spectrum of capensolactone 1. Instead a pair of doublets were present at δ 2.90 and δ 2.50 (J = 5.0 Hz). The HETCOR spectrum showed that both these protons were attached to a carbon atom which resonated at δ 45.7 t in the ^{13}C NMR spectrum. It appeared that the $\Delta^{8(30)}$-double bond had been oxidised to an epoxide as in the ekebergolactones [5]. Further evidence for the 8,30-epoxide came from the mass spectrum. The loss of CH_2O to give a $[M-30]^+$ peak appears typical of compounds with the 8,30-epoxide. H-5 occurred as a double doublet (δ 2.95 $J_{5,6A}$ = 6.6 Hz, $J_{5,6B}$ = 2.7 Hz and H-6A and H-6B occurred as double doublets at δ 2.72 and δ 2.34, respectively.

The typical ekebergin-type ring D was indicated by the C-16 lactone carbonyl resonance at δ 166.4s in the ^{13}C NMR spectrum. Singlets at δ 6.18 and δ 5.71 in the ^{1}H NMR spectrum corresponded to H-17 β and H-15 α respectively. The corresponding carbon resonances occurred at δ 82.0 d and δ 68.8 d, respectively. Compounds from *Ekebergia* commonly occur as the 15 β-acetate. Resonances at δ 168.3 s and 20.3 q were ascribed to carbon atoms of the acetate group. As H-15 α occurred as a singlet, C-14 had to be joined to an oxygen atom. Resonances at δ 75.7 d and 84.7 s ascribable to C-1 and C-14 indicated the presence of the ekebergin-type 1,14-ether linkage.

The COSY spectrum showed coupling between three resonances at δ 4.60 d, 5.25 m and 3.56 d ascribable to H-1 β, H-2 β, H-3 β as is common in compounds from *Ekebergia*. From the chemical shift of H-1 β and H-2 β, the 2-methyl propionate ester was placed at C-2 α, and a hydroxy group at C-3 β. C-2 and C-3 resonated at δ 67.4 d and 76.0 d, respectively.

The structure of ring C remained to be determined. A pair of coupled doublets occurred at δ 5.07 (J = 7.6 Hz) and δ 2.99 in the ^{1}H NMR spectrum. On addition of D_2O, the doublet at δ 2.99 and a singlet at δ 4.41 disappeared and the doublet at δ 5.07 collapsed to a singlet. The doublet at δ 5.07 shifted downfield to become a singlet at δ 5.31 on acetylation, the doublet at δ 2.99 disappeared and the second hydroxy proton resonance shifted to δ 4.20, indicating that one hydroxy group had acetylated but an unacetylated tertiary hydroxy group remained. An acetate methyl group three proton singlet appeared at δ 1.89 in the ^{1}H NMR spectrum of the acetylated product. This upfield resonance is typical for compounds acetylated at C-12 α

[6]. C-12 occurred as a doublet at δ 74.5 in the ^{13}C NMR spectrum. The keto group (δ 208.4 *s*) and tertiary hydroxy group still had to be placed. In limonoids with the 11-keto, 12α-acetate arrangement, H-12 β occurs as a singlet at δ 6.45[9]. This was not the case with capensolactone 1 acetate, thus it appeared that capensolactone 1 had the rearranged ring C structure as in the ekebergolactones. Thus structure **1** is proposed for capensolactone 1. In compounds with the cyclopentanoid ring C and keto group at C-9, C-10 resonates in the δ 54–57 range. In ekebergin type compounds, C-10 occurs further upfield in the δ 35–40 range.

Connolly [7] postulated that the trijugins may be formed by ring C contraction occurring by a pinacol-pinacolone rearrangement of a 9,11-dihydroxy precursor. In capensolactone 1, ring contraction of a 9-hydroxy-11 keto precursor would have to occur to give the 11-hydroxy product.

Capensolactone 2 was isolated as a mixture with esters interchanged at C-2 α and C-3 α. This is common with complex limonoids of the ekebergin-type [5]. Mass spectroscopy revealed a molecular ion at *m/z* 838. An ion at $[M-30]^+$ indicated that the 8,30-epoxide was also present in these compounds. The compounds were diacetates with acetate groups at C-15 β and C-12 α. H-15 α occurred at δ 5.80 *s* and H-12 β at δ 5.29 as in capensolactone 1 acetate. The upfield acetate methyl proton resonance at δ 1.80 confirmed the presence of the acetate group at C-12 α. The COSY spectrum showed H-1 β, H-2 β, H-3 β to occur at δ 4.71 (d), 5.73 (m) and 5.25 (m), respectively. The chemical shifts of H-2 β and H-3 β indicated that esters were present at C-2 β and H-3 β. The complexity of the H-2 β and H-3 β resonances compared to those of capensolactone 1 resonances indicated that these compounds were mixed esters at C-2 α and C-3 α.

From the ^1H NMR spectrum it was evident that the two esters present in ring A were a nicotinate and a 2-methylbutyrate. The presence of the nicotinate ester was confirmed by an ion at *m/z* 106 in the mass spectrum and resonances at δ 9.20 (H-2′), δ 8.78 (H-6′) δ 8.24 (H-4′) and δ 7.43 (H-5′) in the ^1H NMR spectrum. The corresponding carbon resonances occurred at δ 151.6, δ 154.1, δ 138.8, δ 141.0 and the ester carbonyl carbon resonance occurred at δ 165.1 *s*. The presence of the 2-methyl butyrate ester was indicated by a peak at $[M-101]^+$ in the mass spectrum and resonances at δ 2.55 (*m*, H-2″), 1.31 (*m*, H-3″), 1.13 (*d*, H-5″) and 0.63 (*t*, H-4″). The corresponding ^{13}C NMR resonances occurred at 40.0 *d*, 27.4 *t*, 20.5 *q* and 10.8 *q*. Thus structures **2a** and **2b** are proposed for the two isomers of capensolactone 2.

Mass spectroscopy of capensolactone 3 indicated a molecular formula of $C_{41}H_{47}O_{15}N$. Ring B was opened to give a carbomethoxy group at C-7 (3H-7, δ 3.67) and a $\Delta^{8(30)}$-double bond shown by resonances at δ 5.09 and δ 5.31 for the non-equivalent H-30 protons. A ring D lactone with a 15 β-acetate group (3H, δ 2.16) was present as in capensolactones 1 and 2. The complexity of the H-2 β and H-3 β resonances showed that these compounds occurred as a mixture of interchanged esters at C-2 α and C-3 α. NMR and mass spectroscopy indicated that the esters present were nicotinate and 2-methylpropionate. An acetate group was present at C-12 α (3H, δ 1.91) and H-12 β occurred as a doublet at δ 5.69. H-12 β was coupled to a doublet ascribable to H-11 at δ 3.36 (J = 3.1 Hz). H-11 showed long range coupling to 2H-30. No H-9 was present, indicating a 5-membered ring C. A keto group (δ 204.5) was present at C-9 as in capensolactone 2. The value of 3.1 Hz for $J_{11,12}$ indicated a *trans*-relationship between H-11 and H-12, a model showing a dihedral angle of 100° between H-11 α and H-12 β. Thus structures **3a** and **3b** were assigned to the two isomers of capensolactone 3. Limonoids with the contracted ring C, $\Delta^{8(30)}$-double bond and ester at C-15 β have not been reported previously.

The results of the brine shrimp bioassay are shown in Table 2. Solutions of 10 μ g/ml^{-1}, 100 μ g/ml^{-1} and 1000 μ g/ml^{-1} of the crude hexane extract after removal of oils, capensolactone 1 and the capensolactone 2 mixture of esters were used and the method of McLaughlin [10] was employed. The tests demonstrated moderate biological activity of the compounds.

EXPERIMENTAL

NMR spectra were obtained in $CDCl_3$ with TMS as an internal standard. NMR spectra were recorded on a Varian Gemini 300 spectrometer. ^{13}C NMR data are given in Table 1. IR spectra were recorded on a Nicolet Impact 400 FT-IR. Melting points (uncorrected) were determined on an Ernst Leitz Wetzlar mp apparatus. Optical rotations were measured with a Perkin-Elmer 241 digital polarimeter. CC was performed on Si gel 9385 (230–400 mesh, E. Merck, Darmstadt, Germany) and flash chromatography on Si gel 7729 (finer than 230 mesh, E. Merck, Darmstadt, Germany). All solvents were spectral grade or redistilled before use.

Toxicity testing. Testing of crude fractions and compounds was performed using the brine shrimps lethality test according to ref. [10].

Collection and extraction of plant material. The seeds of *Ekebergia capensis* were collected in the Eastern Transvaal in February 1994 and identified by us. Voucher specimens (e.c.s.1) are deposited in the herbarium of the Organic Chemistry Research Group, University of Natal, Durban, South Africa. Air-dried peeled seeds (225 g) were powdered and extracted successively with hexane, $CHCl_3$ and MeOH in a Soxhlet apparatus. CC of the precipitate from the hexane extract using as an eluent $CHCl_3$. EtOAc mixtures, yielded two pure compounds, methyl 3 α-hydroxy-3-deoxyangolensate and capensolactone 1 and capensolactones 2 and 3 which occurred as mixed esters at C-2 α and C-3 α.

Methyl 3 α-hydroxy-3-deoxyangolensate **5**. m.p.

Table 1. ^{13}C NMR data for methylangolensate, **5**, and capensolactones 1–3 (**1-3**) (75 MHz, $CDCl_3$)

Carbon atom	**5**	**1**	**2**	**3**
1	75.3 *d*	75.7 *d*	75.1 *d*	77.3 *d*
2	39.7 *t*	67.4 *d*	67.0 *d*	66.1 *d*
3	73.1 *d*	76.0 *d*	74.2 *d*	77.3 *d*
4	40.2 *s*	39.9 *s*	39.7 *s*	39.9 *s*
5	39.5 *d*	36.2 *d*	35.6 *d*	35.3 *d*
6	28.6 *t*	29.7 *t*	28.4 *t*	28.7 *t*
7	175.4 *s*	176.7 *s*	178.9 *s*	177.2 *s*
8	147.1 *s*	59.2 *s*	59.4 *s*	126.6 *s*
9	51.8 *d*	208.4 *s*	206.7 *s*	204.5 *s*
10	41.0 *s*	55.1 *s*	56.9 *s*	52.7 *s*
11	32.3 *t*	83.5 *s*	82.9 *s*	69.1 *d*
12	29.7 *t*	74.5 *d*	75.0 *d*	76.8 *d*
13	46.7 *s*	54.1 *s*	53.8 *s*	56.4 *s*
14	79.3 *s*	84.7 *s*	84.3 *s*	89.0 *s*
15	32.3 *t*	68.8 *d*	68.6 *d*	69.3 *d*
16	170.2 *s*	166.4 *s*	166.5 *s*	165.8 *s*
17	79.3 *d*	82.0 *d*	79.3 *d*	79.2 *d*
18	15.8 *q*	10.1 *q*	11.0 *q*	12.3 *q*
19	20.3 *q*	19.8 *q*	19.6 *q*	20.8 *q*
20	121.0 *s*	120.7 *s*	119.9 *s*	120.4 *s*
21	140.9 *d*	140.3 *d*	140.5 *d*	140.3 *d*
22	110.1 *d*	109.1 *d*	108.9 *d*	108.4 *d*
23	142.8 *d*	143.1 *d*	143.1 *d*	143.0 *d*
28	22.8 *q*	28.3 *q*	27.0 *q*	22.9 *q*
29	25.9 *q*	22.2 *q*	23.1 *q*	27.7 *q*
30	117.4 *t*	45.7 *t*	45.3 *t*	116.6 *t*
$-OCH_3$	51.7 *q*	52.3 *q*	52.1 *q*	52.0 *q*
$COCH_3$	—	168.3 *s*	168.1 *s*	169.0 *s*
$COCH_3$	—	—	169.7 *s*	169.6 *s*
—	—	20.3 *q*	20.0 *q*	20.5 *q*
—	—	—	19.8 *q*	20.5 *q*
$COCH(CH_3)_2$	—	173.7 *s*	—	173.8 *s*
$COCH(CH_3)_2$	—	33.3 *d*	—	33.6 *d*
$COCH(CH_3)_2$	—	21.3 *q*(×2)	—	18.5 *q*(×2)
CONic	—	—	165.1 *s*	165.3 *d*
CONic	—	—	154.1	152.7 *d*
—	—	—	150.3 *d*	150.3 *d*
—	—	—	141.0 *d*	139.7 *d*
—	—	—	138.8 *d*	136.0 *d*
—	—	—	122.7 *s*	123.2 *s*
$COCH(CH_3)Et$	—	—	176.9 *s*	—
CH_2CH_3	—	—	27.4 *t*	—
CH_2CH_3	—	—	10.8 *q*	—
$CH(CH_3)Et$	—	—	40.0 *d*	—
$CH(CH_3)Et$	—	—	20.5 *q*	—

Note: Values within columns may be interchanged.

203–207°, $[\alpha]_D^{20} = -43$ ($CHCl_3$, *c* 0.356), (14.2 mg). EIMS: $[M]^+$ at *m/z* 472.2400, (calcd for $C_{27}H_{36}O_7$ 427.2461). IR ν_{max}^{KBr} cm^{-1}: 3490, 1720, 1680. ^1H NMR: (300 MHz, $CDCl_3$) δ 0.79, 0.83, 0.92, 1.23 (tertiary methyls) 3.64 (CO_2Me), 4.95, 5.10 (2H-30), 5.66 (H-17), 6.35 (H-22) 7.36, 7.37 (H-21, H-23).

Capensolactone 1 (**1**). (10.1 mg); EIMS: M^+ at *m/z* 678.0888 $g.mol^{-1}$, (calcd for $C_{33}H_{42}O_{15}$ 678.2680), 648, 630, 607; m.p. 217–220°; $[\alpha]_D^{20} = +10.8$ ($CHCl_3$, *c* 0.238); IR ν_{max}^{NaCl} cm^{-1}: 3460, 1770, 1690; ^1H NMR (300 MHz,$CDCl_3$) δ 7.54 (*s*, H-21), 7.40 (*s*, H-23), 6.57 (*s*, H-22), 6.18 (*s*, H-17), 5.71 (*s*, H-15), 5.25 (*dd*, $J = 3.7, 2.3$ H-2 β), 5.07 (*d*, $J = 7.6$, H-12 β), 4.60 (*d*, $J = 3.7$, H-1 β), 4.41 (*s*, 11-OH) 3.64 (3H, *s*, OCH_3), 3.56 (*d*, $J = 2.3$, H-3 β), 2.99 (*d*, $J = 7.6$, 12-OH) 2.95 (*dd*, $J = 2.7, 6.6$, H-5), 2.90 (*d*, $J = 5.0$, H-30a), 2.83

Table 2. Brine shrimp broassay. Deaths (%) after 24 hours

	10 μg ml^{-1}	100 μg ml^{-1}	1000 μg ml^{-1}
Crude hexane extract (after removal of oils)	10	39	61
Capensolactone 1	10	45	74
Capensolactone 2	10	48	80

(sept, J = 7.1, $(CH_3)_2$-C*H*), 2.72 (*dd*, J = 6.6, 18.4, H-6a), 2.50 (*d*, J = 5.0, H-30b), 2.34 (*dd*, J = 2.7, 18.4, H-6b), 2.14 (3H, *s*, $OCOCH_3$), 1.24 (6H, *d*, $(CH_3)_2CH$) 1.21, 1.11, 1.06, 0.97 (4 × CH_3, *s*, H-18, 19, 28, 29).

Capensolactone 2 (**2a** *and* **2b**). (7.3 mg). EIMS: M^+ *m/z*; 838, 824, 808, 796, 766, 754, 738, 724, 106; IR ν_{max}^{NaCl} 3440, 1745 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.20, 8.78, 8.24, 7.43 (4H, H-2′, H-6′, H-4′, H-5′ Nicotinate ester), 7.64 (H-21), 7.45 (H-23), 6.49 (H-22), 5.80 (*s*, H-15 α), 5.73 (*m*, H-2 β), 5.29 (*s*, H-12 β), 5.25 (*m*, H-3 β), 4.71 (*d*, J = 3.9, H-1 β), 3.64 (3H, *s*, OCH_3), 3.34 (*dd*, J = 2.3, 6.5, H-5), 2.82 (*d*, J = 5.0, H-30a), 2.69 (*dd*, J = 2.3, 19.2, H-6a), 2.57 (d, J = 5.0, H-30b) 2.55 (*m*, H-2′)2.33 (dd, J = 6.5, 19.2, H-6b), 2.16, 1.80 (ea3H, *s* ($OCOCH_3$), 1.31 (2H, *m*, CH_3CH_2), 1.13 (3H, *d*, J = 6.2, (CH_3-CH), 0.63 (3H, *t*, J = 7.5, CH_3CH_2), 1.60, 1.46, 1.18, 0.89 (4 × CH_3, *s*, H-18, 19, 28, 29).

Capensolactone 3 (**3a** *and* **3b**). (3.3 mg); EIMS: M^+ *m/z* 793, ($C_{41}H_{47}O_{15}N$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.09, 8.48, 8.09, 6.93 (4H, H-2′, 6′, 4′, 5′, nicotinate ester), 7.40 (*s*, H-23), 7.22 (*s*, H-21), 6.21 (*s*, H-22), 6.07 (*s*, H-17 β) 5.82 (*s*, H-15 α), 5.69 (*d*, J = 3.1, H-12 β), 5.51 (*m*, H-2 β), 5.37 (*m*, H-3 β), 5.31 (*d*, J = 1.1, H-30a), 5.09 (*d*, J = 1.1, H-30b), 4.32 (*m*, H-1 β), 3.67 (3H, *s*, -OCH_3), 3.36 (*d*, J = 3.1, H-11 α), 3.28 (*dd*, J = 2.5, 6.8, H-5), 2.87 (*dd*, J = 2.5, 18.3, H-6a), 2.71 (*sept*, J = 7.0, $(CH_3)_2CH$), 2.28 (*dd*, J = 6.8, 18.3, H-6b), 1.91, 2.16 (3H, *s*, $OCOCH_3$), 1.21 (6H, *d*, J = 6.5, $(CH_3)_2CH$), 1.20, 1.19, 0.97, 0.92 (4 × CH_3, *s*, H-18, 19, 28, 29). IR ν_{max}^{NaCl} cm^{-1}: 3432, 1780, 1720, 1690, 1439, 1375, 1291, 1220, 1081, 1018, 758.

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