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A new lignan and a new sesquiterpene from *Eurotia ceratoides* (L.)

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ABSTRACT

A new lignan, rayalinol (**1**) and a new sesquiterpene kairatene (**2**) were isolated from *Eurotia ceratoides* (L.) along with four hitherto unreported syringaresinol, dehydrodiconiferyl aldehyde, dihydrodehydrodiconiferyl alcohol, and dehydrodiconiferyl alcohol and two previously reported constituents ferulic acid and β-sitosterol. The structures of the new constituents were elucidated by extensive spectroscopic means and chemical evidences. Rayalinol is a biogenetically interesting compound with a hitherto unreported 9a homolignane skeleton. Its biosynthetic origin is suggested.

1. Introduction

The republic of Kazakhstan is a rich vegetative resource, which can be used both for medicine and for agriculture purposes. One such plant, namely *Eurotia ceratoides* (L.) was selected for the present study. The genus *Eurotia* comprises a total of seven species in Central Asia out of which two *E. ceratoides* (L.) and *E. eversmanniana* (Stschegl), are found in Kazakhstan.[1,2] Various groups of workers have undertaken studies on the chemical constituents of this plant and reported different classes of compounds, which include vanillic acid and its derivatives, flavanoids, sterols,[3] caffeic acid and its derivatives.[4]

In the present study we isolated two new constituents, rayalinol (**1**) and kairatene (**2**), together with six known natural products, syringaresinol,[5] dehydrodiconiferyl aldehyde,[6] dihydrodehydrodiconiferyl alcohol,[7] dehydrodiconiferyl alcohol,[8] ferulic acid,[9] and β-sitosterol β-D-glucoside.[10] The structures of **1** and **2** (Figs. 1 and 2) were determined by spectroscopic analysis and chemical transformation, whereas the known compounds were identified through comparison of their physical data with those reported in literature.

2. Result and discussion

The molecular formula of compound **1** was established, through HREIMS, which showed the molecular ion peak at *m/z* 418.1616. The IR spectrum displayed absorption bands attributable to a hydroxyl group (3474 br, cm^{-1}) and a benzene ring (1592.4–1424.2 cm^{-1}). The UV spectrum showed absorption maxima at 235.6 and 273.6 nm. The molecular formula and a careful analysis of the NMR data of **1** collectively suggested that it was a 9a-homolignane [11] belonging to 7′-*epi*-series.[12,13] The ^{13}C NMR spectrum showed 22 carbon atoms, which were distinguished by DEPT experiments as three methyls, two methylenes, ten methines and seven quaternary carbon atoms. In the ^{1}H NMR spectrum signals were present for two CH groups at δ_H 3.05 and 3.11, each one- proton multiplet (H-8, H-8′), two benzylic OCH protons at δ_H 4.11 (d, J=6.3 Hz, H-7) and 4.76 (br.s, C-7′), and one carbinylic CH at δ_H 4.97 (m, H-9). Further, two oxygenated CH_2 groups showed up at δ_H 4.31 (dd, J=11.6,

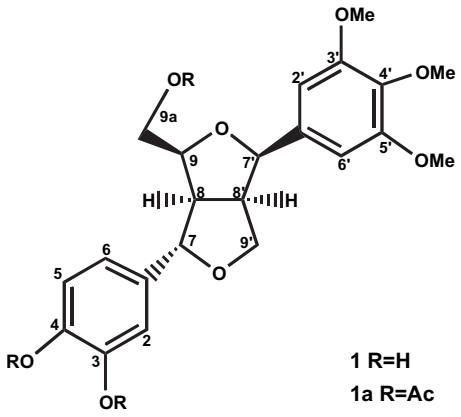

Figure 1. Rayalinol (**1**).

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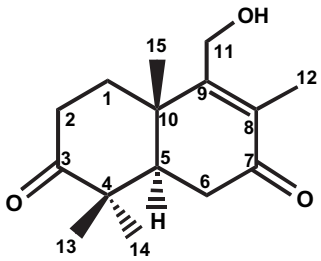


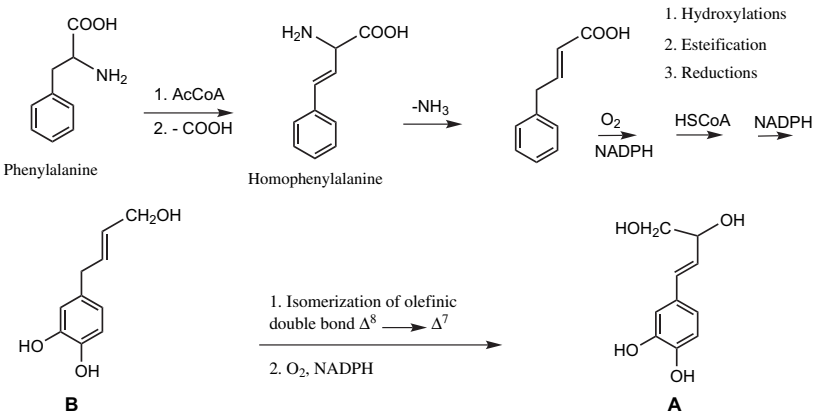
Figure 2. Kairatene (**2**).

6.3 Hz, H-9′a) and 3.96 (dd, *J*=11.6, 8.7 Hz, H-9′b), and at δ_H 3.89 (dd, *J*=12.0, 6.5 Hz, H_a-9a) and 3.46 (dd, *J*=12.0, 6.5 Hz, H_b-9a). A trisubstituted benzene ring was manifested by signals at δ_H 6.94 (br s H-2), 6.83 (d, *J*=8.0 Hz, H-5) and 6.72 (br d, *J*=8.0 Hz, H-6) and a tetra-substituted benzene ring by a two- proton singlet at δ_H 6.62 (br s, H-2′, H-6′). The upfield shift of one of the benzylic protons (δ_H 4.11, H-7) was in agreement with its axial nature, which was due to a direct anisotropic field effect of an axial aryl group in the opposite ring (i.e., at C-7′), while the other benzylic proton (H-7′) resonated more downfield due to being equatorial.[12,13] The COSY spectrum showed two structural units in the furo-furan part, one comprising –O–^{9a}CH$_2$–^{9}CH(O)–^{8}CH–^{7}CH–and the other one O–$^{9'}$CH$_2$–$^{8'}$CH–$^{7'}$CH(O)– consistent with an additional carbon (C-9a) in the lignan skeleton. Its position was further confirmed by HMBC correlations between H-9 and C-7, C-8, and C-9a, and between both H_a-9a, H_b-9a and C-8 and C-9. The NOESY spectrum displayed correlations of H-7′ with H-8′ and H-9, and of H-7 with both H_a-9a and H_b-9a revealing that H-7′, H-8′ and H-9 were arranged in the same face of the furofuran ring. The relatively downfield appearance of H-9 was attributed to the long-range anisotropic deshielding of the equatorially oriented benzene ring at C-7.[14] The presence of three hydroxyl groups in the molecule was confirmed by acetylation of **1**, yielding a triacetate **1a** which, in the ^{1}H NMR spectrum, showed one aliphatic acetoxy methyl resonating at δ_H 1.96, and two aromatic acetoxy groups resonating at δ_H 2.12 and 2.22 (Table 1). The further deshielding of H-9 (C δ_H 6.06) in **1a** was due to the deshielding anisotropy of the acetyl carbonyl at γ-position.[15] A comparison of the chemical shifts with values reported in literature revealed the presence of a trimethoxyphenyl ring.[16] Its placement at C-7′ was established by HMBC correlations of H-7′ with C-1′, and C-2′/C-6′. It should further be noted that the upfield shift of C-1′ (δ_C 131.2) is in agreement with an axially placed aryl ring at C-7′ as compared to that of C-1 (δ_C 134.3) due to an equatorial aryl ring at C-7.[12] The pronounced downfield shift of δ_H 4.11 (H-7) compared to axial benzylic protons in other furofuran type lignans[12,13] was attributed to its 1,3-diaxial interaction with the axially oriented -CH$_2$OH at C-9.[15] The remaining two hydroxyl groups required by the molecular formula were positioned at C-3 and C-4 of the second aryl ring on the basis of NOESY interactions in **1a** between the two acetoxy methyls resonating at δ_H 2.19 and 2.22. In the light of these data, the structure of **1** was identified as 3′, 4′, 5′- trimethoxy-7, 9′:7′, 9-diepoxy-lignan-3, 4, 9a- triol, representing a new natural product for which the name rayalinol is proposed.

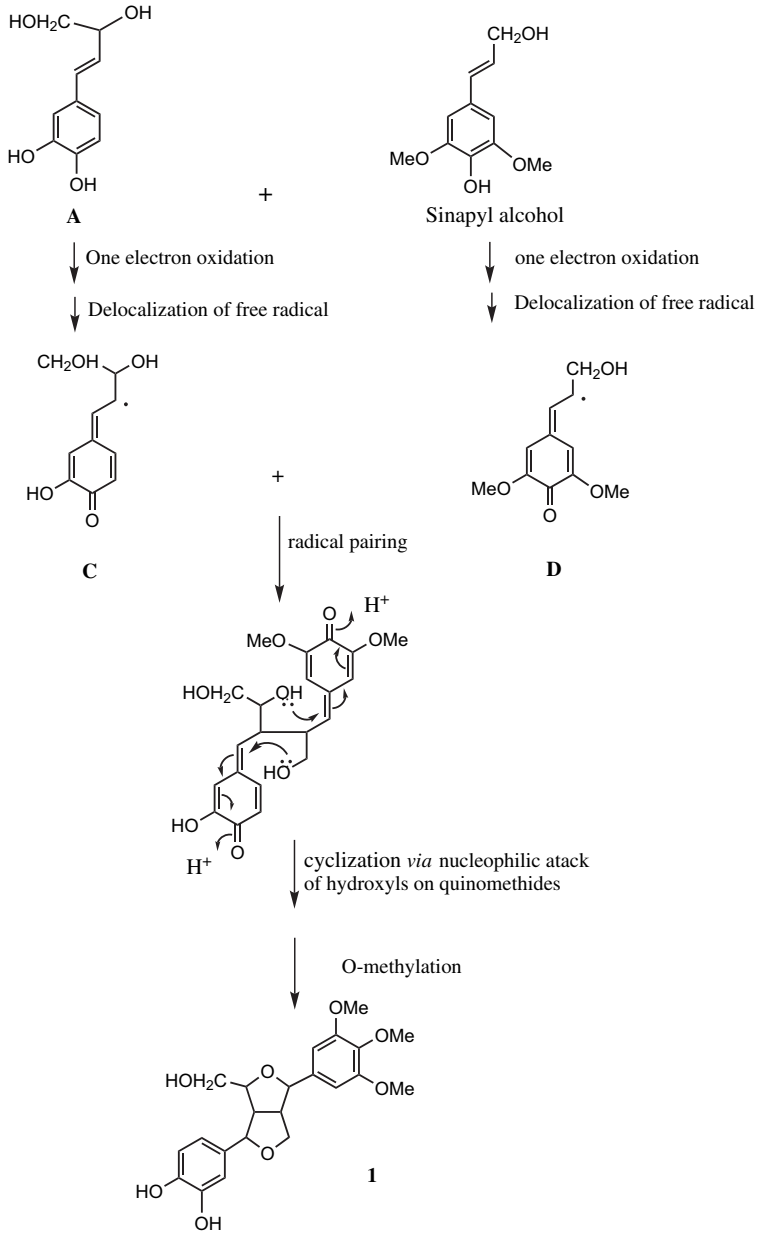
Rayalinol (**1**) is a biogenetically interesting compound with a hitherto unreported 9a-homolignan skeleton. Lignans are known to form from two phenylpropanoid (C_6–C_3) monomers (cinnamic acids) derived from phenylalanine, whereas in **1**, one of the monomers involved is its homologue (C_6–C_4 unit). It is proposed that this homologation may occur through the well known chain elongation of amino acids[17] like that of methionine to homomethionine[18] and of phenylalanine to homophenylalanine[19] by their deamination to the respective ketoacids, condensation with acetate, loss of original carboxyl group and *re*-amination. Homophenylalanine thus formed may undergo the biochemical processes similar to those reported[17] for the formation of cinnamyl alcohols from L-phenylalanine, to afford B, which is the Δ^8 isomer of the required alcohol. Isomerization of the double bond from C-8 to C-7 and oxidation at C-9 would afford A (Scheme 1). One-electron oxidation[17,20] of the phenol A as well as of sinapyl alcohol by peroxidase enzyme and delocalization of the unpaired electron into the side chain would yield the reactive quinomethides C and D, respectively. Their radical pairing followed by intramolecular cyclization[17,20], and O-methylation would finally provide rayalinol (Scheme 2).

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1** and **1a** in ($CDCl_3$)

1					**1a**	
	δ_C	δ_H (*J* in Hz)	HMBC	NOESY	δ_C	δ_H (*J* in Hz)
1	134.3	—			136.1	—
2	108.3	6.94, br s	1, 3, 4, 6	9′a	122.4	6.95, m
3	146.6	—			150.7	—
4	144.8	—			139.3	—
5	114.1	6.83, d (8.0)	1, 4, 6		111.3	6.93,d (8.0)
6	118.7	6.72, br d (8.0)	1, 2, 4, 5	8,8′	119.0	6.88, br d (8.0)
7	87.0	4.11 (br s)	1, 2, 6, 8, 9	9a, 9b	80.8	4.58, m
8	54.5	3.05 (br s)	1, 7, 9, 9a	9,7′	54.3	3.04, br s
9	72.5	4.97 (br s)	7′, 8, 9a	7′, 8	73.8	6.06, br s
9a-H_a	60.5	3.89, dd (12.0, 4.0)	8, 9	7	62.6	4.43, dd (11.2, 6.0)
9a-H_b		3.46, dd (12.0, 9.0)	8, 9	7		4.21, dd (11.2, 3.5)
1′	131.2	—			132.0	—
2′	102.5	6.62, s	1′, 3′, 4′, 6′	9′a, 9′b	102.6	6.49, br s
3′	153.3	—			153.3	—
4′	137.6	—			137.2	—
5′	153.4	—			153.3-	—
6′	102.5	6.62, s	1′, 3′, 4′, 6′	9′a, 9′b	102.6 (br s)	6.50, br s
7′	85.8	4.76, br s	1′, 2′, 8′, 9′	8′, 9	85.8	4.69, d (6.0)
8′	54.4	3.11, br s	1′, 7′, 9′	7′, 8	54.4	3.04, d (6.0)
9′a	72.0	4.31, dd (11.6, 6.3)	7, 7′, 8′	8′	72.0	4.25, dd (10.5, 6.2)
9′b		3.96, dd (11.6, 4.7)	7, 7′, 8′			3.89, dd (10.5, 4.0)
OMe	55.9, 56.2×2	3.87×3			56.0×2	3.72
					55.9	3.78
OAc		—			20.7	1.96
OAc		—			20.8	2.22
OAc		—			21.1	2.19



Scheme 1.



Scheme 2.

The molecular formula of **2** was evident from the molecular ion peak at *m*/*z* 250.1582 in the HREIMS spectrum, which was in accordance with $C_{15}H_{22}O_3$. The IR spectrum displayed absorption bands attributable to a hydroxyl group (3473 cm^{-1}), a carbonyl in six- membered ring (1732 cm^{-1}) and an α,β-unsaturated ketone (1680 cm^{-1}). The UV spectrum showed absorption maxima at 221.6 and 237.8 nm. The molecular formula and the ^{13}C NMR spectrum (Table 2) suggested that **2** was a sesquiterpenoid containing four methyls including one vinylic methyl, four methylenes, one methine and six quaternary carbons including two olefinic and two carbonylic carbons. The ^1H NMR (Table 2), and 2D *J*-resolved spectra manifested two spin systems $-^1CH_2-^2CH_2-$ and $-^5CH-^6CH_2-$. The HMBC correlations (Table 2) were particularly helpful in placing the isolated ketone (δ_C 214.6) at C-3 and the other carbonyl at C-7 in conjugation with the double bond at C-8. In addition, there were present two downfield AB doublets at δ_H 4.38 and 4.32 (each 1H, d, *J*=11.6, H-11a, H-11b) due to carbinylic methylene protons next to a C=C.[21] Further, the HMBC correlations between H-11a and H-11b with C-8, C-9 and C-10 confirmed its location at C-9. A vinylic methyl group resonating at δ_H 1.88 had correlations in the HMBC spectrum with C-7, C-8 and C-9 showing its position at C-8. The *trans* A/B ring junction could be decided on the basis of coupling constants of H-5 and NOESY interactions (Table 2). Thus the *J*-values of H-5 showed its axial disposition, and its interaction with H-14 in the NOESY spectrum revealed that both are on the same plane of the molecule and hence 14-CH_3 is equatorial. On the other hand, NOESY correlations between H-13 and H-15 suggested that both these have same (axial) disposition which is *trans* to H-5. These data led to assign the structure of **2** as 3–7-dioxodrim-8-en-11-ol, a new natural product for which the name kairatene is proposed. **2** represents a dimarane sesquiterpenoid and its biogenesis may be envisaged from dimarenol through enzymatic oxidations at C-3 and C-7 and migration of the double bond from C-7 to the more stable conjugated position C-8.[22,23]

Table 2
^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **2** (in $CDCl_3$)

No	δ_C	δ_H (*J* in Hz)	HMBC	NOESY
1α	39.0	1.50, ddd (13.5, 10.0, 6.5)	2, 3, 10,15	5
1β		1.95, ddd (13.5, 4.5, 3.2)	2, 3, 5, 10, 15	15
2α	34.2	2.51, ddd (13.5, 5.5, 2.2)	1, 3, 4, 10	14
2β		2.43, ddd (13.5, 10.0, 4.5)	1, 3, 4, 10	13, 15
3	214.6	—		
4	46.8	—		
5	49.1	2.21, dd (10.0, 4.7)	3, 4, 6, 7, 10	1α, 14
6α	35.4	2.68, dd (14.0, 7.5)	5, 7	5
6β		2.54, dd (14.0, 10.0)	5, 7, 8	13, 15
7	199.0	—		
8	133.0	—		
9	160.0	—		
10	39.1	—		
11a	58.8	4.38, d (11.6)	8, 9, 10	1β, 12, 15
11b		4.32, d (11.6)	8, 9, 10	
12	11.3	1.88, s	7, 8, 9	11
13	21.0	1.11, s	3, 4, 5	2β, 6β, 15
14	26.4	1.44, s	3, 4, 5	2α, 5, 6α
15	17.9	1.23, s	1, 5, 9, 10	1β, 2β, 6β, 13

3. Experimental section

3.1. General experimental procedures

Column chromatography was carried out by using silica gel 60 (Merck, 70–230 mesh). TLC was carried out on silica gel 60 PF_{254} (Merck), VLC on silica gel 9385 (Merck) with detection by UV at 254, 366 nm, and by staining with I_2 vapour. UV spectra were obtained using a Hitachi-U-3200 spectrophotometer. IR spectra were recorded on a Jasco A-302 spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured on Bruker AMX-500 spectrophotometer. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference. Mass spectra were obtained using Finnigan-Mat 311A spectrometer, at 70 eV. Preparative HPLC was carried out on a JAI LC-908 W system equipped with a 3 mL injection loop. Normal phase silica gel column from Japan Analytical Instruments (JAI) was used; dimension: 20 cm×250 nm.

3.2. Plant material

The aerial parts (stem and leaves) of *E. ceratoides* (L.) were collected from the Ili region during 2005 and identified by Miss. Zarina Inelova, Department of Botany, al-Farabi Kazakh National University, and a voucher specimen (NO: EC-214) has been deposited in the herbarium of the same department.

3.3. Extraction and isolation

10 kg of *Eurotia ceratoides* (L.) were chopped to small pieces and extracted with MeOH (30 L×4) at room temperature. The combined extract was concentrated under vacuum to give a thick syrup, which was partitioned between EtOAc (4.0 L) and water (2.5 L). The resulting EtOAc phase was dried over anhydrous Na_2SO_4 and passed through a charcoal bed, which was washed with EtOAc (500 mL) and MeOH–benzene (1:1, 250 mL). The combined organic phases were concentrated under vacuum, and the resulting yellowish brown residue was treated with petroleum ether (PE) to give PE soluble (residue=49 g) and insoluble fractions (25 g). The insoluble fraction was subjected to VLC (petroleum ether, petroleum ether–EtOAc, $CHCl_3$, $CHCl_3$–MeOH in increasing order of polarity). 60 fractions were obtained and combined on the basis of TLC to ultimately afford 14 fractions (A-N). Fraction B (PE–EtOAc; 9:1 eluate) afforded pure β-sitosterol-β-D-glucoside (4.2 mg), fraction C (PE–EtOAc; 8.5:1.5 eluate) afforded pure ferulic acid (5.5 mg). Fraction D (300 mg, eluated with 100% $CHCl_3$) was further passed through a silica gel column using $CHCl_3$ as a mobile phase with gradually increasing polarity by adding MeOH. As a result, nineteen fractions were collected, and fraction 19 (143 mg; eluated with $CHCl_3$–MeOH 9.3:0.7) was separated by preparative HPLC with 5% isopropyl alcohol in $CHCl_3$ as mobile phase. Four peaks were collected and identified as syringaresinol (6.7 mg, T_R 22 min), rayalinol (**1**; 12.3 mg, T_R 28.7 min), dehydrodiconiferyl aldehydes (3.9 mg, T_R 40 min) and dihydrodehydrodiconiferyl alcohol (2.1 mg, T_R 43 min). Fraction G was purified through preparative TLC ($CHCl_3$–MeOH; 9.5:0.5) and furnished kairatene (**2**) as an amorphous powder (3.0 mg).

Fraction F (250 mg, also $CHCl_3$ eluate) was further passed through a silica gel column using $CHCl_3$ as a mobile phase with gradually increasing polarity by adding MeOH. As a result ten fractions were collected. Fraction 6 (100 mg; eluated with $CHCl_3$–MeOH; 9.5:0.5) was separated through preparative HPLC with 7% isopropyl alcohol in $CHCl_3$ as mobile phase yielding dehydrodiconiferyl alcohol (5.0 mg, T_R 36 min).

3.4. Characteristics of new compounds

*3.4.1. Rayalinol (**1**).* Amorphous solid; $[\alpha]_D^{25}$ +0.05 (*c* 0.039); UV (MeOH) λ_{max} (log ε) 235.6 (7.34), 273.6 (6.94) nm; IR (KBr) ν_{max} 3474 br, 1592.4–1424.2 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 418.1616 [$M^{+\bullet}$] (calcd for $C_{22}H_{26}O_8$, 418.1620). EIMS *m*/*z* (rel.int): 418 (M^+, 19), 210 (16), 180 (64), 137 (100), 121 (12).

*3.4.2. Kairatene (**2**).* Amorphous solid; $[\alpha]_D^{25}$ −0.5 (*c* 0.6); UV (MeOH) λ_{max} (log ε) 221.6 (7.36), 237.8 (6.97) nm; IR (KBr) ν_{max} 3473, 1732.2, 1680 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 250.1582 [$M^{+\bullet}$] (calcd for $C_{15}H_{22}O_3$, 250.1563). EIMS

m/*z* (rel.int): 250 (M^+, 11), 219 (100), 189 (7), 177 (20), 163 (23), 111 (44).

*3.4.3. Acetylation of rayalinol (**1**).* To a solution of **1** (5 mg) in pyridine (0.5 mL) acetic anhydride (0.5 mL) was added and the reaction mixture left overnight at room temperature. It was poured over crushed ice and extracted with ethyl acetate. The ethyl acetate phase was washed with water, dried (anhyd Na_2SO_4), and freed of the solvent to give an amorphous solid (4.5 mg). EIMS *m*/*z* (rel.int): 544 ($M^{+\bullet}$, 5), 502 (7), 484 (4), 460 (7), 387 (4), 179 (100), 131 (90). ^1H NMR and ^{13}C NMR data, see Table 1.

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