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NEO-CLERODANE-TYPE DITERPENOIDS FROM *SALVIA KEERLII**

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Key Word Index—*Salvia keerlii*; Labiatae; diterpenoids; neo-clerodanes, kerlin; kerlinolide.

Abstract—From the aerial parts of *Salvia keerlii* two new neo-clerodane diterpenoids have been isolated. Their structures: 8,12(*R*)-epoxyneo-cleroda-3,13(14)-diene-18,19:15,16-diolide, kerlin and 7-acetoxy-12(*R*)-hydroxyneo-clerodane-3,13(14)-diene-18,19:15,16-diolide, kerlinolide, have been established by spectroscopic means. X-ray diffraction analysis was performed for kerlin.

INTRODUCTION

Salvia sp. (Labiatae) are abundant in Mexico [T. P. Ramamoorthy, personal communication]. Some of these plants are used in folk medicine [1, 2], as hallucinogens [3] or as culinary herbs. A variety of bi- and tri-cyclic diterpenes have been isolated from these species which show antifeedant, antitumour or antifungal properties. In a previous study of *S. melissodora* we isolated [4] melisodoric acid (**1**) which showed antifeedant activity.

Salvia keerlii Bentham (*Salvia* section, *Scorodonia*, Epling) seems to be related to *S. melissodora* Lag, from which it is distinguished by its glandular calyx [T. P. Ramamoorthy, personal communication].

RESULTS AND DISCUSSION

From the aerial parts of this plant two new diterpenes were isolated to which we assigned structures **2** and **4a** (R = H) from the following considerations.

Kerlin (**2**), showed a $C_{20}H_{24}O_5$ molecular formula. Its IR spectrum (ν_{max}) presented a strong absorption at 1764 cm^{-1}, which was ascribed to the α,β-unsaturated γ-lactone attached to the A ring. A weak absorption at 1785 cm^{-1} and a strong one at 1750 cm^{-1} could be due to the Fermi resonance [5] shown by the β-substituted butenolide function [6] present in structure **2**. Two bands at 1675 and 1637 cm^{-1} were assigned to the conjugated double bonds. The UV spectrum [λ_{max} nm (ε): 208 (17 540)] supports these assignments.

The ^{1}H NMR spectrum of kerlin (**2**) (Table 1) showed a double doublet (1H, J = 3.5 and 6 Hz) centred at δ6.7 which could be attributed to an olefinic β-proton of an α,β-unsaturated γ-lactone group coupled to a methylene moiety, therefore, it was assigned to H-3. It also showed the typical signals of a β-substituted butenolide ring: a double doublet at δ5.9 (1H J = 2 and 1 Hz) and a doublet at δ4.8 (2H, J = 2Hz) which could be assigned unambiguously to the H-14 vinylic proton and to the CHz-16 group by double resonance experiments. The H-14 proton was also coupled to H-12 (J = 1 Hz) which appeared as a double, double doublet at δ4.85 (J = 4, 8 and 1 Hz). Irradiation at δ4.8 transformed the signal at δ5.9 into a sharp singlet. The chemical shift shown by H-12 can be explained if it is geminal to an ethereal oxygen atom and also allylic. The ^{1}H NMR spectrum of kerlin (**2**) did not show the Me-17 doublet usually found [1, 7, 8] in diterpenes of the labdane–clerodane family. Therefore the ethereal oxygen must be attached to C-8. The Me-17 appeared as a singlet at δ1.1. The H-11 protons were found at δ2.55 (*dd*, J = 4 and 12 Hz) and 1.85 (*dd*, J = 8 and 12 Hz) by irradiation at δ4.85.

The two H-19 protons are responsible for an AB system observed at δ4.25 and 3.85 (J = 8 Hz). The doublet at δ3.85 showed a long-range coupling (J = 1 Hz) as has been described [9, 10] for clerodan-18,19-olides lacking a substituent at C-6.

The ^{13}C NMR spectrum (Table 2) of kerlin was in agreement with the structure and relative stereochemistry shown in **2**, with the exception of the C-12 chiral centre which could not be assigned with the data presented. Assignments were based on data described for similar neo-clerodane structures [6–11].

Catalytic hydrogenation of kerlin (**2**) gave the tetrahydro derivative (**3**) which showed, in the IR spectrum (ν_{max}), a sharp absorption at 1775 cm^{-1} due to the saturated γ-lactones produced. Its ^{1}H NMR spectrum did not show the presence of any vinylic protons but, instead, a complex signal at δ3.75–4.40(5H) could be assigned to the protons on carbons bearing oxygen atoms. The Me-20 and Me-17 groups appeared as two singlets (3H each) at δ0.8 and 1.10, as in the parent compound **2**.

The second product isolated from *S. keerlii*, kerlinolide, showed a molecular formula $C_{22}H_{28}O_7$. Structure **4a** was proposed for it, based on biogenetic grounds and spectroscopic data. Its IR spectrum (ν_{max}) showed a hydroxyl absorption at 3450 cm^{-1}. A weak absorption at 1760 cm^{-1} and a strong, wide band at 1740 cm^{-1} was assigned to the α,β-unsaturated γ-lactone function bound to ring A, the β-substituted butenolide ring and an ester function. Two weak bands at 1668 and 1636 cm^{-1} were attributed to the conjugated double bonds.

The ^{1}H NMR spectrum of **4a** (Table 1) showed, in the methyl region, a singlet at δ0.85 and doublet at δ0.95 (J = 7 Hz) assigned to the Me-20 and Me-17 groups. It also

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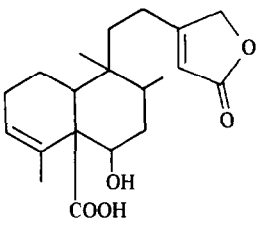
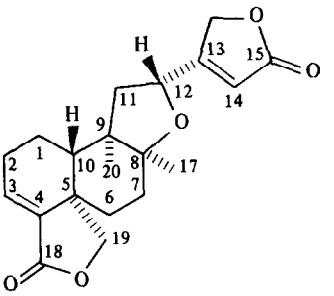


1



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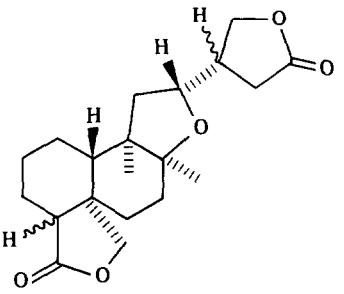

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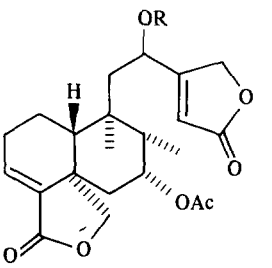

4a R = H
4b R = Ac

showed the presence of a β-substituted butenolide function [δ5.9 (1H, *dt*, $J=1$ and 2 Hz) and 4.9 (2H, *d*, $J=2$ Hz)]. The vinyllic H-3 was responsible for a double doublet observed at δ6.7 (1H, $J=3$ and 6 Hz). A singlet at δ2.1 was attributed to an acetate group whose geminal proton was responsible for a double triplet (1H, $J=2$ and 4 Hz) centred at δ5.25. The acetate group, therefore, must be bound to a secondary carbon atom flanked by methyne and methylene groups. Irradiation of the signal at δ5.25 transformed a complex signal centred at δ2.55 into a quartet ($J=7$ Hz). This signal could be assigned to H-8 as irradiation of the secondary methyl doublet simplified it into a doublet ($J=4$ Hz). The acetate group, therefore, must be attached to C-7 and must be axially orientated since its geminal proton showed coupling constants ($J=2$ and 4 Hz) due to equatorial–equatorial and equatorial–axial interactions. The axial acetate group at C-7 produces a strong deshielding effect on H-19β which was observed as a doublet ($J=10$ Hz) at δ4.85; the H-19α was responsible for a double doublet (1H, $J=10$ and 1 Hz) observed at δ3.90. The long-range coupling suggested the absence of substituents at C-6.

The hydroxyl group observed in the IR spectrum of **4a** was responsible for a broad doublet (1H, $J=4$ Hz) found at δ3.15 which was lost on addition of deuterated water; its geminal proton was clearly observed as a broad doublet (1H, $J=10$ Hz) at δ4.65. On acetylation of **4a**, this signal was shifted downfield and appeared as a broad doublet at δ5.60 (1H, $J=10$ Hz) revealing its allylic nature. The hydroxyl group in **4a**, therefore, must be attached to C-12 as in olearin [10] and marrubiastrol [6].

The ^{13}C NMR spectrum of **4a** (Table 2) was in agreement with the structure and relative stereochemistry proposed for it. The *R*-configuration assigned to C-12 was based on a possible biogenetic relationship of **4a** with kerlin.

The *R*-configuration, assigned to C-12 in kerlin, was found by X-ray analysis of a single crystal, which confirmed the proposed structure and stereochemistry.

The molecular structure together with the numbering scheme is illustrated in Fig. 1. The cyclohexene ring exhibits a puckered sofa conformation and it is *trans*-fused to the cyclohexane ring which has a chair conformation. The Me-17 and Me-20 groups occupy equatorial and axial sites, respectively. The five-membered ring bridge to the cyclohexane through the C-8–C-9 bond and the γ-lactone ring, adopt the envelope conformation. The conformation of the 13(16H)-furanone ring can best be described as a flattened envelope with C-13 as the flap. The arrangement of molecules in the crystal appears to be determined by Van der Waals interactions.

EXPERIMENTAL

Mps are uncorr. MS were obtained by direct inlet at 70 eV. ^{1}H NMR and ^{13}C NMR spectra were performed at 80 and 20 MHz, respectively, in $CDCl_3$ soln or DMSO with TMS as int. standard. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant material was collected in July 1982, near Matatlán (Oaxaca, México), and a voucher specimen (MEXU-320784) was deposited at the herbarium of the Instituto de Biología, U.N.A.M.

Isolation of kerlin. Dried and powdered aerial parts of *S. keerlii*

Table 1. ^{1}H NMR spectral data of compounds **2**, **3**, **4a** and **4b***

	2	**3**	**4a**	**4b**
H-3	6.7 *dd* (3.5, 6)	†	6.7 *dd* (3, 6)	6.75 *dd* (4, 6)
H-7β	†	†	5.25 *dt* (2, 4)	5.27 *dt* (2, 4)
H-8	†	†	2.55 *dq* (7, 4)	†
H-11β	2.55 *dd* (12, 4)	†	†	†
H-11α	1.85 *dd* (12, 8)	†	†	†
H-12	4.85 *dd* (8, 4)	3.9 *m* (6, 8)	4.65 *br d* (10)	5.6 *br d* (10)
H-14	4.8 *d* 2H (2, 1)	4.3 *m* 2H	4.9 *d* 2H (2)	4.7 *d* 2H (1)
H-16	5.9 *dd* (2, 1)	—	5.9 *dt* (1, 2)	5.95 *dt* (1, 2)
Me-17	1.1 *s*	1.1 *s*	0.95 *d* (7)	1 *d* (7)
H-19β	4.25 *d* (8)	4.85 *d* (8)	4.85 *d* (8)	4.85 *d* (8)
H-19α	3.85 *dd* (8, 1)	4.20 *d* (8)	3.9 *dd* (8, 1)	3.9 *dd* (8, 1)
Me-20	0.85 *s* 3H	0.8 *s* 3H	0.85 *s* 3H	0.85 *s* 3H
OAc	—	—	2.1 *s* 3H	2.1 *s* 6H

*Chemical shifts are in δ-values from TMS (80 MHz, $CDCl_3$ solution). Coupling constants [J (Hz)] are in parentheses.

†Multiplets between δ 1.25 and 2.5 complex and overlapped signals.

Table 2. ^{13}C NMR chemical shifts of compounds **2** and **4a**

	2	**4a**
C-1	20.45 (*t*)	19.01 (*t*)
C-2	29.76* (*t*)	26.94 (*t*)
C-3	135.93 (*d*)	136.01 (*d*)
C-4	137.85 (*s*)	137.95 (*s*)
C-5	46.67 (*s*)	47.07 (*s*)
C-6	29.52* (*t*)	38.77* (*t*)
C-7	27.29* (*t*)	71.61 (*d*)
C-8	84.70 (*s*)	39.48 (*d*)
C-9	44.44 (*s*)	44.39 (*s*)
C-10	43.65 (*d*)	47.07 (*d*)
C-11	41.97 (*t*)	37.04* (*t*)
C-12	71.02 (*d*)	64.28 (*d*)
C-13	173.12 (*s*)	169.63 (*s*)
C-14	112.44 (*d*)	112.94 (*d*)
C-15	174.60 (*s*)	176.04 (*s*)
C-16	71.02 (*t*)	73.51 (*t*)
C-17	25.50 (*t*)	11.58 (*q*)
C-18	168.44 (*s*)	168.40 (*s*)
C-19	70.92 (*t*)	71.61 (*t*)
C-20	16.12 (*q*)	19.01 (*q*)
OCOMe	—	173.33 (*s*)
OCOMe	—	20.88 (*q*)

In δ-values from TMS (20 MHz, in DMSO-d_6). SFORD multiplicities in parentheses.

*Values in any vertical column may be interchanged but those given here are considered to be most likely.

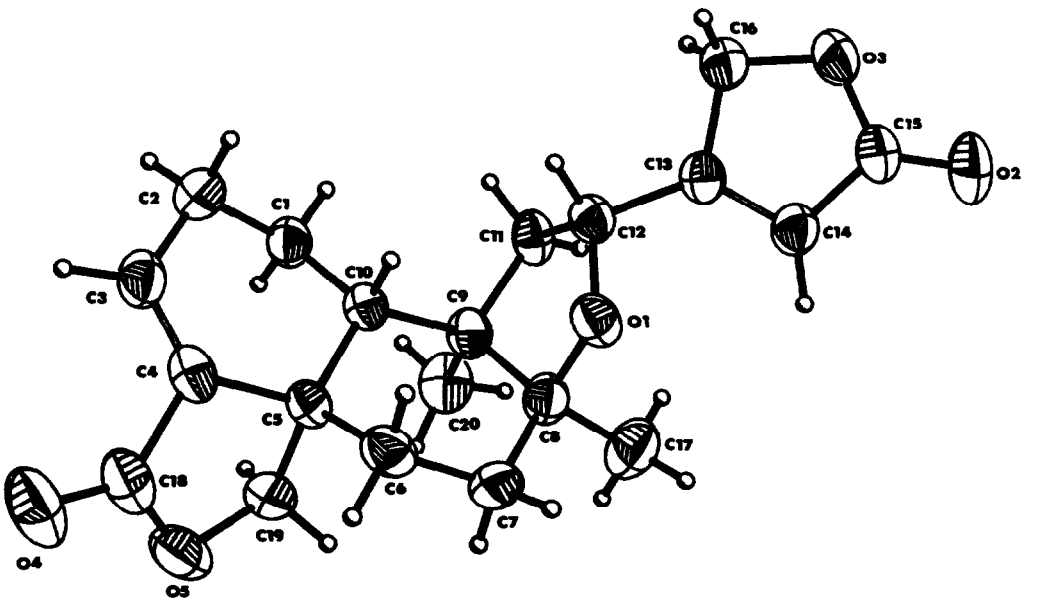

Fig. 1. The molecular conformation of kerlin, showing atom numbering. The thermal ellipsoids are drawn at the 50% probability level.

(100 g) were extracted with Me_2CO at room temp. for 1 week. The solvent was removed under red. pres. and the gummy residue obtained (8.9 g) chromatographed over silica gel (150 g, deactivated with 10% H_2O). Elution with EtOAc–MeOH (9:1) gave kerlin (**2**, 672 mg): mp 220–222° from Me_2CO–hexane; $[\alpha]_D^{20} - 59°$ (MeOH; *c* 0.200); UV λ_{max}^{MeOH} nm (ε): 208 (17 500); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1784, 1764, 1750, 1657, 1637, 1220, 1184, 1144, 1022, 975, 887, 870; ^1H NMR ($CDCl_3$, 80 MHz): Table 1; ^{13}C NMR (DMSO-d_6, 20 MHz): Table 2. MS *m/z* (rel. int.): 344 $[M]^+$ (2.7), 329 (100), 314 (43.4), 300 (8), 217 (10), 201 (20), 180 (35), 105 (20), 91.2 (52.8), 81 (10), 77 (20). ($C_{20}H_{24}O_5$ requires: $[M]^+$ at *m/z* 344).

Catalytic hydrogenation of kerlin. Kerlin (**2**, 50 mg) in MeOH was hydrogenated using Pd–C (5%, 15 mg) as catalyst. After the usual work-up, the non-crystalline product (**3**) showed IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 1775, 1220, 1172, 1010; $[\alpha]_D^{20} + 15.11°$ (MeOH; *c* 0.172); ^1H NMR ($CDCl_3$, 80 MHz): Table 1; MS *m/z* (rel. int.): 333 $[M - 15]^+$ (100), 262 (20), 219 (15), 105 (10), 91 (17), 81 (11), 79 (20). ($C_{20}H_{28}O_5$ requires: $[M]^+$ at *m/z* 348).

Isolation of kerlinolide (**4a**). The mother liquors of **2** were extensively chromatographed on silica gel. Compound **4a** (265 mg) crystallized from Me_2CO–hexane: mp 100–102°; $[\alpha]_D^{20} - 106.6°$ (MeOH; *c* 0.15); UV λ_{max}^{MeOH} nm (ε): 208 (22 000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3453, 1760, 1742, 1658, 1636; ^1H NMR ($CDCl_3$, 80 MHz): Table 1; ^{13}C NMR (DMSO-d_6, 20 MHz): Table 2; MS *m/z* (rel. int.): 344 $[M - 60]^+$ (24), 315 (20), 246 (10), 217 (18), 201 (12), 186 (13), 143 (22), 105 (20), 91 (46.8), 81 (10). [$C_{22}H_{28}O_7$ requires: $[M]^+$ at *m/z* 404 (not observed).]

Acetylation of kerlinolide. Kerlinolide (30 mg) in C_5H_5N (1.0 ml) and Ac_2O (1.0 ml) at room temp. for 2 hr, gave **4b**: mp 220–222°; $[\alpha]_D^{20} - 121°$ ($CHCl_3$; *c* 0.2); UV λ_{max}^{MeOH} nm (ε): 208 (13 750); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1774, 1751, 1660, 1645, 1224, 1034, 871; ^1H NMR ($CDCl_3$, 80 MHz): Table 1; MS *m/z* (rel. int.): 446 $[M]^+$ (0.3), 386 (4.1), 357 (17), 344 (8), 287 (8), 217 (18), 105 (15), 91 (29.4), 43 (100). ($C_{24}H_{30}O_8$ requires: $[M]^+$ at *m/z* 446.)

X-ray structure determination of kerlin. Crystals of kerlin (**2**) ($C_{20}H_{24}O_5$, M_r 344.41) prepared by slow evaporation of Me_2CO–hexane, are orthorhombic, space group $P2_12_12_1$ with $a = 7.517$ (1), $b = 11.051$ (4), $c = 20.647$ (5) Å, $V = 1715.2$ (6) Å, $F(000) = 736$, $\mu = 7.38\ cm^{-1}$ and $d_{calc} = 1.327\ g/cm^3$ for $z = 4$. Graphite monochromatic CuK_α radiation ($\lambda = 1.5418$ Å) was selected to measure the intensity of the 1382 independent reflections for $\theta < 57°$, of which 1330 were observed [$I > 2.5\sigma(I)$], on a crystal of 0.35 × 0.32 × 0.5 mm with the aid of an automatic diffractometer Nicolet R3m, index range h 0/9, k 0/13, l 0/23, ω-scan mode, variable scan speed, scan width 1.0° (θ), two standard reflections (200; 213) monitored every 50 measurements. No intensity decay was observed during the expt. Intensities were corrected for Lorentz and polarization effects and absorption was ignored. The crystal structure was solved by direct methods using SHELXTL [12], using 170|E| > 1.6 and refined by least-squares. In the final refinement, anisotropic thermal factors were used for the non-H atoms and for the H atoms riding on the bonded C atoms with a fixed isotropic temp. factor $U = 0.06\ Å^2$. Function minimized $\Sigma w(\Delta F)^2$ with $w = [\sigma^2(F_0) + 0.00151(F_0)^2]^{-1}$. The final discrepancy indexes are $R = 0.032$ and $wR = 0.050$ for the 1330 observed reflections.* The final difference map has no peaks greater than $\pm 0.2\ e/Å^3$ and the isotropic extinction parameter is $X = 0.0175$.

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*A list of atomic parameters, bond distances and angles, anisotropic thermal parameters, torsion angles and $F_0 - F_c$ tables are deposited at the Cambridge Crystallographic Data Centre, U.K.

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