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Neo-clerodane diterpenoids from *Clerodendrum inerme*

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Abstract

Three neo-clerodane diterpenoids, inermes A, B and 14,15-dihydro-15β-methoxy-3-epicaryoptin, have been isolated from the hexane extract of the leaves of *Clerodendrum inerme* in addition to an epimeric mixture of 14,15-dihydro-15-hydroxy-3-epicaryoptin. Structures of these compounds have been elucidated on the basis of spectral studies.

Keywords: *Clerodendrum inerme*; Verbenaceae; Neo-clerodane diterpenoids

1. Introduction

Several neo-clerodane diterpenoids have been isolated during the last few years from genera like *Ajuga*, *Teucrium*, *Scutellaria* and *Clerodendrum*. Interest in these compounds has been stimulated by their biological activity as antifeedants against some economically important lepidopterous pests (Gebbinck et al., 2002) as well as by their antibacterial activity (Chen et al., 1996). Occurrence of dimeric clerodane diterpene has been reported earlier by Barton et al. (1961) from the species *C. infortunatum*.

Leaves of *Clerodendrum inerme* (L.) Gaertn. mixed in housefly larval diet, are found to reduce puparial weights and inhibit adult emergence (Ahmed et al., 1981). (−)-3-Epicaryoptin isolated from the leaves is responsible for growth inhibition and antifeedant activities in housefly and mosquito (Pereira and Gurudutt, 1990). Recently, we have reported new sterols from *C. inerme* (Pandey et al., 2003). Here in this paper we report the isolation and structure elucidation of three new neo-clerodane diterpenoids, namely inermes A (**1**), inermes B (**2**) and 14,15-dihydro-15β-methoxy-3-epicaryoptin (**3**) found in the hexane extract of aerial parts of *C. inerme*. 14,15-Dihydro-15-hydroxy-3-epicaryoptin (**4**) has also been isolated as an epimeric mixture. The structures of these compounds were investigated by comparison of their ^{1}H and ^{13}C NMR and mass spectral data with those reported for similar compounds earlier.

2. Results and discussion

Compound **1** was isolated by preparative TLC of fractions eluted with hexane-ethyl acetate (60:40) and possessed molecular formula $C_{52}H_{74}O_{19}$ (FABMS), suggesting it to be a dimer of clerodane type diterpenoid. The infra red (IR) spectrum of **1** exhibited significant bands at 3030 cm^{-1} (oxirane ring) and at 1738 and 1253 cm^{-1} (ester groups) (Lin et al., 1989). The proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra indicated it to be a dimeric neo-clerodane type diterpenoid. Characteristic signals corresponding to hexahydrofurofuran unit, the two AB spin systems corresponding to the C4–C18 epoxide and C19 methylene, the C20 methyl singlet and C17 methyl doublet were observed similar

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to those found in neo-clerodanes isolated from *Clerodendrum* (Achari et al., 1992; Rao et al., 1993).

In the ^{1}H NMR spectrum the signals, each integrated for six protons, due to acetate groups were observed at δ 1.94, 1.98 and 2.12 (Rao et al., 1993) indicating the presence of six acetate groups in the dimeric diterpene. The characteristic signals of tertiary methyl groups were observed at δ 0.93 (3H, *s*, H-20) and 0.95 (3H, *s*, H-20′) where as those of secondary methyl groups were observed at δ 0.88 (integrated for six protons) as doublet (Chen et al., 1996). The protons on carbon bearing oxygen appeared at: δ 2.59 (2H, *d*, J = 4.2 Hz, H-18, H-18′), 2.85 (2H, *d*, J = 4.2 Hz, H-18, H-18′); 4.37 and 4.82 (each 2H, AB system, J = 12 Hz, H-19, H-19′); 4.77 (2H, *dd*, J = 11.0, 4.6 Hz, H-6, H-6′) and 5.30 (2H, *m*, H-3, H-3′) (Achari et al., 1992; Lin et al., 1989). Two different sets of ^{1}H NMR values were observed for the hexahydrofurofuran ring moieties of the compound. Signals appeared at δ 4.40 (1H, *dd*, J = 11.3, 5.4 Hz, H-11), 5.08 (1H, *d*, J = 5.7 Hz, H-15), 5.79 (1H, *d*, J = 5.4 Hz, H-16) and 2.95 (1H, *m*, H-13) for the ring having α-substituent at C-15 where as for the ring moiety having β-substituent the signals were observed at δ 4.00 (1H, *dd*, J = 11.9, 4.5 Hz, H-11′), 5.21 (1H, *d*, J = 4.7 Hz, H-15′), 5.71 (1H, *d*, J = 5.4 Hz, H-16′) and 3.00 (1H, *m*, H-13′) (Jannet et al., 1999). From the presence of these signals it could be concluded that there are two hexahydrofurofuran rings in the compound which differ only in the stereochemistry at C-15.

This fact was well supported by the ^{13}C NMR spectrum of compound **1**. It showed signals at δ 103.7 and 109.0 and at δ 103.5 and 107.0 corresponding to C-15 and C-16 of the α and β substituent carrying hexahydrofurofuran ring moieties, respectively (Jannet et al., 1999). Besides these it also showed overlapped signals at δ 169.5, 169.9 and 171.0 for the carbonyl function of the acetate groups. All methyls of the acetate groups appeared at δ 20.9 as overlapped signals (Rao et al., 1993). The carbons bearing the acetate groups resonated at δ 67.1 (C-3), 67.0 (C-3′), 71.2 (C-6), 71.1 (C-6′), 62.7 (C-19) and 63.0 (C-19′). The ring carbon involved in the epoxide ring formation resonated at δ 65.2 (overlapped signals, C-4 and C-4′) where as the methylene group of the epoxide resonated at δ 42.4 (C-18 and C-18′, overlapped signals). The tertiary methyl appeared at δ 13.9 (C-20) and 13.8 (C-20′) where as the secondary methyl appeared at δ 16.2 (C-17) and 15.0 (C-17′). The carbons of both hexahydrofurofuran ring, i.e. C-11 and C-11′, joined to the decalin ring appeared at δ 83.3 (Beauchamp et al., 1996).

Further support to the dimeric nature of the compound was provided by the FAB mass spectral data which showed $[M + H]^+$ peak at *m*/*z* 1003 in accordance with the proposed structure. The base peak appeared at *m*/*z* 111 due to furofuran ring fragment ion formed as a result of cleavage of C-9/C-11 bond and subsequent loss of substituent at C-15 (Chen et al., 1996). The cleavage at C-15′ of the dimer results in the formation of prominent ions at *m*/*z* 493 and 509. Fragment ion peak at *m*/*z* 621 appears due to loss of one decalin ring moiety from **1**.

Thus, on the basis of above it can be concluded that the compound inermes A, a dimeric neo-clerodane having a hexahydrofurofuran ring joined through an ethereal linkage at C-15, has structure **1**.

Later fractions eluted with the same ratio of hexane:ethyl acetate afforded compound **2** by preparative thin layer chromatography. It possessed a molecular formula $C_{53}H_{76}O_{20}$ (*m*/*z* 1033 $[M + H]^+$). The spectroscopic properties of **2** established that this dimeric diterpene is closely related to **1**; the difference of OCH_3 between the molecular formulae of the two diterpenes **1** and **2** and the presence of supplementary signal assignable to a methoxy group in the ^{1}H and ^{13}C NMR spectra of **2** suggested that compound **2** is a methoxy derivative of **1**. The IR spectrum showed similar significant absorptions attributable to oxirane ring (3030 cm^{-1}) and ester groups (1736 and 1253 cm^{-1}) (Lin et al., 1989).

The ^{1}H NMR spectrum of **2** was similar to that of **1**, with the notable exception of a distinctive resonance at δ 3.32 (3H, s) due to methyl function of a methoxy group (Jannet et al., 1999). The methine proton attached to the methoxy group appeared at δ 3.65 (1H, *m*, H-1). Similar to **1,** compound **2** contained signals for the two tertiary methyls at δ 0.92 (3H, *s*, H-20) and 0.95 (3H, *s*, H-20′), two secondary methyl groups at δ 0.87 (6H, *d*, J = 6 Hz, H-17 and H-17′, overlapped signals) and six acetate residues as singlets at δ 1.95, 1.99 and 2.13 (overlapped signals, each signal integrating for six protons). The ^{1}H NMR spectrum showed the two AB quartets, the primary carbinol methylene groups as overlapping signals at δ 4.37 (2H, *d*, J = 12 Hz, H-19 and H-19′) and 4.80 (2H, *d*, J = 12 Hz, H-19 and H-19′) and the epoxide methylene groups at δ 2.59 (2H, *d*, J = 3.9 Hz, H-18 and H-18′) and 2.85 (2H, *d*, J = 3.9 Hz, H-18 and H-18′); a multiplet at δ 5.28 due to C-3 methine protons and a doublet of doublets at δ 4.65 (2H, *dd*, J = 11.0, 4.6 Hz, H-6 and H-6′) due to protons at position 6 (Hosozawa et al., 1974). The presence of two hexahydrofurofuran rings in compound **2** was revealed by the following data. The ring having α-substituent at C-15 displayed signals at δ 4.40 (1H, *dd*, J = 11.3, 5.4 Hz, H-11), 5.02 (1H, *d*, J = 5.7 Hz, H-15), 5.79 (1H, *d*, J = 5.4 Hz, H-16) and 2.90 (1H, *m*, H-13), while the ring having β-orientation displayed signals at δ 4.05 (1H, *dd*, J = 11.9, 4.5 Hz, H-11′), 5.15 (1H, *d*, J = 4.7 Hz, H-15′), 5.68 (1H, *d*, J = 5.4 Hz, H-16′) and 3.01 (1H, *m*, H-13′) (Jannet et al., 1999). Thus, similar to **1** compound **2** is also a dimer formed by C-15 isomers with an additional methoxy group at C-1.

The ^{13}C NMR spectrum of **2** showed resonances, which also corroborated the presence of two clerodane units, joined by an ethereal linkage at C-15 and C-15′. The anomeric carbons of α-substituted ring appeared at δ 103.8 (C-15) and 109.1 (C-16) where as those of β-substituted ring appeared at δ 103.6 (C-15′) and 107.1 (C-16′) (Jannet et al., 1999). The carbons of both the rings involved in oxirane formation resonated at 42.6 (C-18 and C-18′) and 65.3 (C-4 and C-4′). The C-11 and C-11′ resonated at δ 83.1 and 83.4, respectively. The tertiary methyl groups appeared at δ 13.9 (C-20) and 13.8 (C-20′) where as the secondary methyl groups appeared at δ 16.2 (C-17) and 15.1 (C-17′). The ring carbons attached to the acetate groups appeared at δ 67.2 (C-3 and C-3′) and 71.3 (C-6 and C-6′) while the methylene groups attached to the acetate groups appeared at δ 62.8 (C-19) and 63.1 (C-19′) (Beauchamp et al., 1996). Overlapping signals at δ 21.0 and 21.1 appeared due to the methyl function of acetate groups and at δ 169.6, 170.0 and 171.1 due to the carbonyl carbon of the six acetate groups (Rao et al., 1993). The methoxy group in **2** resonated at δ 54.5. The position of the OCH_3 was deduced at carbon 1 (or 1′) due to presence of shifted signals of C-1 (or 1′) at δ 71.1and C-2 at δ 32.7 (Min et al., 1989; Shimomura et al., 1981).

The dimeric nature of **2** was also evident from its FAB mass spectral data which showed $[M + H]^+$ at *m/z* 1033. Occurrence of C-15 substituted hexahydrofurofuran moiety was confirmed by the presence of significant ion at *m/z* 111, resulting from the substituent loss from the furofuran ring fragment ion of a neoclerodane diterpene (Chen et al., 1996; Jannet et al., 1999). Loss of one decalin moiety from the dimer resulted in ion peak at *m/z* 651 where as loss of decalin ring carrying substituent at position 1 (or 1′) from the dimer resulted in ion peak at *m/z* 621. Hence compound **2** contains one additional OCH_3 than **1**. Cleavage at C-15′ of the dimer resulted in the formation of significant fragment ions at *m/z* 493 and 539.

Thus, on the basis of above spectral data structure of inermes B was assigned as **2**.

Compound **3**, obtained by preparative TLC of fractions eluted with hexane:ethyl acetate (25:75), has molecular formula $C_{27}H_{40}O_{10}$ (*m/z* 525 $[M + H]^+$). The IR spectrum revealed the presence of esters (1736 and 1256 cm^{-1}) and an oxirane ring (3030 cm^{-1}) (Lin et al., 1989). Analyses of the ^1H NMR and ^{13}C NMR spectra of **3** indicated clearly that it also belonged to the neo-clerodane diterpenoid class. Furthermore, by direct comparison of the NMR spectra of **3** with that of an earlier reported neo-clerodane derivative (Achari et al., 1992) it was obvious that the compounds contained common functionalities.

The 300 MHz ^1H NMR showed signals due to one tertiary methyl (δ 0.93, 3H, *s*), one secondary methyl group (δ 0.89, 3H, *d*, *J* = 6.4 Hz), three acetate residues (δ 1.95, 1.99 and 2.13, each 3H, *s*) and one methoxy group (δ 3.34, 3H, *s*) (Achari et al., 1992). It also showed the two AB quartets, typical of a primary carbinol methylene group at δ 4.79 and 4.37 (2H, *J* = 12 Hz, H-19), and an epoxide methylene group at δ 2.84 and 2.61 (2H, *J* = 4 Hz each, H-18); a doublet of doublets at δ 4.76 (1H, *J* = 11.1, 4.6 Hz) due to a C-6 proton; and a multiplet at δ 5.28 due to C-3 proton (Hosozawa et al., 1974). Signals at δ 4.03 (1H, *m*, H-11), 5.12 (1H, *d*, *J* = 5 Hz, H-15), 5.73 (1H, *d*, *J* = 5.4 Hz, H-16) and 3.02 (1H, *m*, H-13) indicated the presence of a β-substituent carrying hexahydrofurofuran ring in the compound (Jannet et al., 1999).

The structure of compound **3** was further confirmed by its ^{13}C NMR spectrum. A lowfield ^{13}C NMR resonance at δ 104.4 and a methoxyl group resonance at δ 54.1 suggested the substitution of a H-15 proton by a methoxyl group (Chen et al., 1996). Besides these the spectrum contained signals at δ 13.3 and 15.8 due to the C-20 and C-17 methyl groups, respectively. Signals attributable to the three acetate groups appeared at δ 169.5, 170.0 and 171.2, and at δ 20.4 (3 signals overlapped), where as the signals due to carbons bearing the acetate groups appeared at δ 61.0 (C-19), 66.8 (C-3) and 71.0 (C-6). The carbons involved in the oxirane ring formation resonated at δ 42.1 (C-18) and 64.8 (C-4). Other significant signals were observed at δ 82.9 (C-11) and 106.8 (C-16) (Beauchamp et al., 1996).

The above NMR assignments were also substantiated by FAB mass spectral data. $[M + H]^+$ ion was observed at *m/z* 525. Significant ion peak at *m/z* 143 with the base peak at *m/z* 111 arises via cleavage of the C-9/C-11 bond and loss of a molecule of methanol thereafter (Achari et al., 1992).

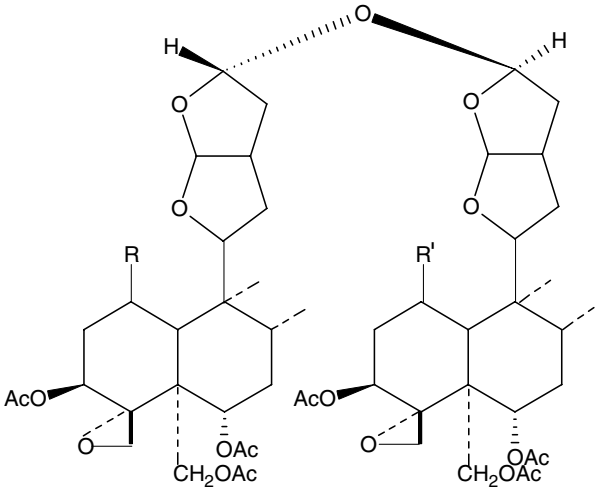
A similar neo-clerodane derivative carrying a C-15 methoxy substituent has been isolated from this plant earlier (Achari et al., 1992) and it has been assigned β-orientation by the authors. However, according to Jannet et al. (1999) its stereochemistry at C-15 should be reversed as its NMR spectral data agrees well with that for C-15α substituted neo-clerodanes and should now be 14,15-dihydro-15α-methoxy-3-epicaryoptin. On this basis compound **3** can be said to be an C-15β epimer of earlier reported compound after comparing the ^1H and ^{13}C NMR spectral data. Thus compound **3** is 14,15-dihydro-15β-methoxy-3-epicaryoptin.

Compound **4**, eluted from fractions with hexane:ethyl acetate (25:75) was isolated by preparative TLC. The structure of **4** clearly followed from its ^1H NMR data, which were closely comparable to those of **3**. However in the ^1H NMR spectrum of **4** the signal of the methoxy group at δ 3.34 was absent. Furthermore, a hydroxyl absorption band was apparent in the IR spectrum of **4** which was absent in **3**. FAB MS suggested its molecular formula as $C_{26}H_{38}O_{10}$. The IR spectrum showed absorption's attributable to free hydroxyl (3454 cm^{-1}), oxirane ring (3030 cm^{-1}) and ester (1732 and 1251 cm^{-1}) groups

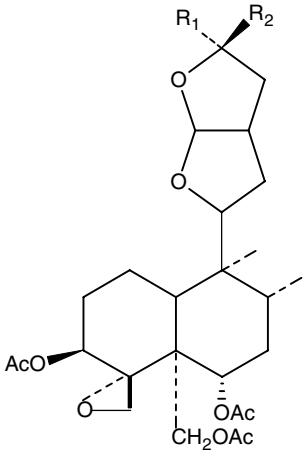
(Chen et al., 1996). The ^{1}H NMR spectrum data of compound **4** was similar to compound **3** differing only in the values of hexahydrofurofuran ring moiety. On the basis of NMR spectral data compound **4** appears to be a mixture of the C-15 epimers of 14,15-dihydro-15-hydroxy-3-epicaryoptin. Protons H-16, H-15 and H-11 appeared as pairs of signals: H-16, δ 5.79 (m) and 5.65 (m); H-15, δ 5.63 (m) and 5.24 (m), and H-11, δ 4.39 (m) and 4.00 (m). Signals corresponding to C-16 appeared at δ 109.5 and 107.4 where as those for C-15 appeared at δ 98.4 and 98.6. Thus ^{1}H and ^{13}C NMR data were in complete agreement (Bruno et al., 2000; Ohno et al., 1996) with the proposed structure (**4**).

Its FAB mass spectrum showed $[M + H]^+$ at *m/z* 511. The significant peaks at *m/z* 129 and 111 resulted from C-9/C-11 bond cleavage and subsequent loss of water molecule thereafter (Chen et al., 1996).

Compound **4** is similar to earlier reported 15-hydroxy epicaryoptin (Kumari et al., 2003). However the name should be revised as 14, 15-dihydro-15-hydroxy-3-epicaryoptin. Thus, compound **4** was assigned as a mixture of C-15 epimers of 14, 15-dihydro-15-hydroxy-3-epicaryoptin.



1 R, R' = H
2 R, R' = OMe, H / H, OMe



3 R_1 = H, R_2 = OMe
4 R_1, R_2 = OH, H / H, OH

3. Experimental

3.1. General

The 300 MHz NMR spectra were recorded in $CDCl_3$ with tetramethyl silane (TMS) as internal standard. The ^{13}C NMR (broad band and DEPT) spectra were recorded at 75 MHz. The DEPT experiments were used to determine the multiplicities of carbon atoms. Ready-made preparative layer silica gel glass plates (Merck, Germany) were used for preparative TLC and the spots were visualized by exposure to I_2 vapours or by spraying with vanillin:sulphuric acid:ethanol (1 g:5 ml:95 ml) reagent followed by heating the plate at 110 °C for 15 min.

3.2. Plant material

Aerial parts of *Clerodendrum inerme* were collected from Lucknow in October 2000 and a voucher specimen (No. CIMAP – 8199) has been deposited in the herbarium of this institute.

3.3. Extraction and isolation

Air dried and finely powdered aerial parts of the plant (8.2 kg) were extracted in a similar manner as reported earlier (Pandey et al., 2003). Elution was carried out in varying percentage of EtOAc in hexane. Fractions eluted with hexane–EtOAc (40:60) afforded compounds **1** and **2**. Compounds **1** (0.010 g) and **2** (0.007 g) were obtained as viscous mass by PTLC using C_6H_6:Me_2CO (80:20) as mobile phase. Compounds **3** and **4** were obtained from later fractions eluted with hexane:EtOAc (25:75). PTLC using C_6H_6:Me_2CO (70:30) as mobile phase yielded **3** (0.009 g) and **4** (0.008 g) as viscous mass.

3.4. Inermes A (*1*)

Viscous mass; $[\alpha]_D$ −18.6° ($CHCl_3$); IR ν_{max} cm^{-1}: 3030, 2947, 1738, 1455, 1375, 1253, 1071, 1036, 756; ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$): see Table 2; FABMS *m/z* (rel. int.): 1003 $[M + H]^+$ (2), 621 (1), 509 (3), 493 (22), 433 (8), 373 (11), 313 (18), 111 (100).

3.5. Inermes B (2)

Viscous mass; $[\alpha]_D$ −12.6° ($CHCl_3$); IR ν_{max} cm^{-1}: 3030, 2938, 1736, 1450, 1367, 1253, 1072, 1004, 868; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$): see Table 2; FABMS *m/z* (rel. int.): 1033$[M + H]^+$ (3), 651 (5), 621 (1), 539 (44), 523 (2), 509 (1), 493 (33), 111 (100).

Table 1
^1H NMR chemical shifts (in δ) for compounds **1**–**4** (in $CDCl_3$)

H	**1**		**2**		**3**	**4**
	H	H′	H	H′		
H-1			3.65, *m*			
H-3	5.30, *m*	5.30, *m*	5.28, *m*	5.28, *m*	5.28, *m*	5.28, *m*
H-6	4.77, *dd*, *J* = 11.0, 4.6 Hz	4.77, *dd*, *J* = 11.0, 4.6 Hz	4.65, *dd*, *J* = 11.0, 4.6 Hz	4.65, *dd*, *J* = 11.0, 4.6 Hz	4.76, *dd*, *J* = 11.1, 4.6 Hz	4.76, *dd*, *J* = 11.1, 4.6 Hz
H-11	4.40, *dd*, *J* = 11.3, 5.4 Hz	4.00, *dd*, *J* = 11.9, 4.5 Hz	4.40, *dd*, *J* = 11.3, 5.4 Hz	4.05, *dd*, *J* = 11.9, 4.5 Hz	4.03, *m*	4.39, *m* and 4.00, *m*
H-13	2.95, *m*	3.00, *m*	2.90, *m*	3.01, *m*	3.02, *m*	3.10, *m*
H-15	5.08, *d*, *J* = 5.7 Hz	5.21, *d*, *J* = 4.7 Hz	5.02, *d*, *J* = 5.7 Hz	5.15, *d*, *J* = 4.7 Hz	5.12, *d*, *J* = 5 Hz	5.63, *m* and 5.24, *m*
H-16	5.79, *d*, *J* = 5.4 Hz	5.71, *d*, *J* = 5.4 Hz	5.79, *d*, *J* = 5.4 Hz	5.68, *d*, *J* = 5.4 Hz	5.73, *d*, *J* = 5.4 Hz	5.79, *d*, *J* = 5.4 Hz and 5.65, *m*
H-18	2.59, *d*, *J* = 4.2 Hz	2.59, *d*, *J* = 4.2 Hz	2.59, *d*, *J* = 3.9 Hz	2.59, *d*, *J* = 3.9 Hz	2.61, *d*, *J* = 4.0 Hz	2.60, *d*, *J* = 4.0 Hz
	2.85, *d*, *J* = 4.2 Hz	2.85, *d*, *J* = 4.2 Hz	2.85, *d*, *J* = 3.9 Hz	2.85, *d*, *J* = 3.9 Hz	2.84, *d*, *J* = 4.0 Hz	2.83, *d*, *J* = 4.0 Hz
H-19	4.37, *d*, *J* = 12 Hz	4.37, *d*, *J* = 12 Hz	4.37, *d*, *J* = 12 Hz	4.37, *d*, *J* = 12 Hz	4.37, *d*, *J* = 12 Hz	4.37, *d*, *J* = 12 Hz
	4.82, *d*, *J* = 12 Hz	4.82, *d*, *J* = 12 Hz	4.80, *d*, *J* = 12 Hz	4.80, *d*, *J* = 12 Hz	4.79, *d*, *J* = 12 Hz	4.79, *d*, *J* = 12 Hz
H-20	0.93, *s*	0.95, *s*	0.92, *s*	0.95, *s*	0.93, *s*	0.94, *s*
H-17	0.88, *d*, *J* = 6 Hz	0.88, *d*, *J* = 6 Hz	0.87, *d*, *J* = 6 Hz	0.87, *d*, *J* = 6 Hz	0.89, *d*, *J* = 6.4 Hz	0.87, *d*, *J* = 5 Hz
$C\underline{H}_3CO$	1.94, 1.98, 2.12, *s*	1.94, 1.98, 2.12, *s*	1.95, 1.99, 2.13, *s*	1.95, 1.99, 2.13, *s*	1.95, 1.99, 2.13, *s*	1.94, 1.98, 2.12, *s*
$C\underline{H}_3O$			3.32, *s*		3.34, *s*	

Table 2
^{13}C NMR spectral data (in δ) for compounds **1**–**4** (in $CDCl_3$)

Carbon no.	**1**		**2**		**3**	**4**
	C	C′	C	C′		
C-1	21.0	21.0	71.1	21.0	21.0	21.1
C-2	30.8	30.8	32.7	30.9	30.5	30.9
C-3	67.1	67.0	67.2	67.2	66.8	67.1
C-4	65.2	65.2	65.3	65.3	64.8	65.3
C-5	46.2	46.2	46.3	46.3	46.0	46.3
C-6	71.2	71.1	71.3	71.3	71.0	71.3
C-7	33.1	33.1	32.3	32.3	32.8	33.1
C-8	35.9	35.9	36.1	35.9	35.4	36.0
C-9	40.1	39.9	40.2	40.0	39.5	40.2
C-10	47.5	47.7	47.6	47.8	47.5	47.6
C-11	83.3	83.3	83.1	83.4	82.9	83.5
C-12	32.6	32.1	32.6	32.2	32.3	32.5
C-13	40.6	40.6	40.7	40.5	40.1	40.9
C-14	39.9	38.1	39.5	38.2	37.6	39.9
C-15	103.7	103.5	103.8	103.6	104.4	98.4 and 98.6
C-16	109.0	107.0	109.1	107.1	106.8	109.5 and 107.4
C-18	42.4	42.4	42.6	42.6	42.1	42.5
C-19	62.7	63.0	62.8	63.1	61.0	61.3
C-20	13.9	13.8	13.9	13.8	13.3	13.8
C-17	16.2	15.0	16.2	15.1	15.8	16.2
$OCO\underline{C}H_3$	20.9 × 3	20.9 × 3	21.0, 21.1	21.0, 21.1	20.4 × 3	20.9 × 3
$O\underline{C}OCH_3$	169.5, 169.9, 171.0	169.5, 169.9, 171.0	169.6, 170.0, 171.1	169.6, 170.0, 171.1	169.5, 170.0, 171.2	169.5, 170.0, 171.1
$O\underline{C}H_3$			54.5		54.1	

3.6. 14,15-Dihydro-15β-methoxy-3-epicaryoptin (**3**)

Viscous mass; $[\alpha]_D$ −57.4° ($CHCl_3$); IR ν_{max} cm^{-1}: 3030, 2936, 1736, 1459, 1383, 1256, 1071, 1041, 754; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$): see Table 2; FABMS *m/z* (rel. int.): 525 $[M + H]^+$ (79), 143 (42), 111 (100).

3.7. 14,15-Dihydro-15-hydroxy-3-epicaryoptin (**4**)

Viscous mass; $[\alpha]_D$ −37.9° ($CHCl_3$); IR ν_{max} cm^{-1}: 3454, 3030, 1732, 1369, 1251; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$): see Table 2; FABMS *m/z* (rel. int.): 511$[M + H]^+$ (100), 129 (40), 111 (80).

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