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ABIETANE DITERPENE ACIDS AND OTHER CONSTITUENTS FROM THE LEAVES OF *LARIX KAEMPFERI*

REIKO TANAKA, HIRONORI OHTSU and SHUNYO MATSUNAGA*

Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-11, Japan

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Key Word Index—*Larix kaempferi*; Pinaceae; *Larix* sp; leaves; sesquiterpene; diterpene; abieta-8,11,13,15-tetraen-18-oic acid; 16-*nor*-15-oxodehydroabietic acid; 12, 15-dihydroxydehydro-abietic acid.

Abstract—The new diterpene acids were isolated from the leaves of *Larix kaempferi*, together with two known sesquiterpenes, (−)-α-cadinol and oplopanone, and 10 known diterpenes, larixol, dehydroabietinol, 7-oxo-dehydroabietinol, 7-oxodehydroabietic acid, 9α,13α-epidioxyabiet-8(14)-en-18-oic acid, 9β,13β-epidioxyabiet-8(14)-en-18-oic acid, dehydroabietic acid, 7α-hydroxydehydroabietic acid, 8α,9α,13α,14α-diepoxyabietan-18-oic acid and 15-hydroxydehydroabetic acid. The new acids and their methyl esters were characterized as methyl abieta-8,11,13,15-tetraen-18-oic acid, methyl 16-*nor*-15-oxodehydroabietic acid and methyl 12,15-dihydroxydehydroabietic acid, on the basis of chemical and spectroscopic evidence. © 1997 Elsevier Science Ltd

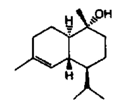
INTRODUCTION

In the course of a search for biological active constituents from the leaves and bark of coniferous trees which have been treated as wastes in the forestry industry, we found that several tetracyclic triterpenes and their derivatives isolated from the stem bark of some *Abies* species exhibit a potent anti-tumour-promoting activity against two stage skin-tumour induced mice without any toxicity [1]. As a part of the above study, we examined a *Larix* species which are the only deciduous members of the Pinaceae. Previous workers had reported the isolation of abietic acid from the oleoresins of some larch [2], 13-epimanool from *L. decidua* [3], larixyl 6-acetate from *L. europea* [4], cembrane and larixol from *L. sibilica* [5]. *L. kaempferi* (Lamb.) Carr. (*L. leptolepis* Murray, Japanese name: Karamatsu) is a tall tree indigenous to Japan and is now widely distributed not only within the subalpine belts of this country but also in China and central Europe by afforestation [6]. The oleoresin of this tree is used as a raw material for telebin oil production [7]. A literature survey revealed that larixol, larixyl diacetate, 3β-hydroxyepimanool, 15-oxopimara-8(14)-en-18-oic acid, 7-oxo-15-hydroxydehydroabietic acid, 15-hydroxydehydroabietic acid and 7-oxo-13-hydroxyabieta-8(14)-en-18-oic acid were present in the oleoresin of *L. kaempferi*, as well as cupressic acid and 3β-hydroxysandaracopimaric acid from that of Kamchatka larch (*L. gmelini*) and methyl 8,15-dihydroxyabiet-13-en-18-oate from that of *L. sibirica* [8]. Furthermore, a considerable number of mono- and sesqui-terpenes including α-cedrene are biosynthesized from mevalonate in callus cultured from the seedling leaves of *L. kaempferi* [9], together with two lignans [10]. Detailed examination of the methylene chloride extract of the leaves of *L. kaempferi* has now led to the isolation of three new diterpene acids, together with two known sesquiterpenes and 10 known diterpenes.

RESULTS AND DISCUSSION

Preliminary silica gel column chromatography of the dichloromethane extract of the leaves of *L. kaempferi* afforded both neutral and acidic fractions. Repeated column chromatography of the neutral fraction furnished two known sesquiterpene alcohols, (−)-α-cadinol (**1**) [11] and oplopanone (**2**) [12], and three known diterpene alcohols, dehydroabietinol (**3**) [13], 7-oxodehydroabietinol (**4**) [14] and larixol (**5**) [15]. Treatment of the acidic fractions with diazomethane diethyl ether and subsequent column chromatography of the resulting products furnished three new diterpene acids, **13**, **14** and **15**, as the corresponding methyl esters, **13a–15a**, together with seven known diterpene acids, 7-oxodehydroabietic acid (**6**) [16], 9α,13α-epidioxyabiet-8(14)-en-18-oic acid (**7**), [17], 9β,13β-epidioxyabiet-8(14)-en-18-oic acid (**8**) [17], dehydroabietic acid (**9**) [18], 7α-hyd-

* Author to whom correspondence should be addressed.



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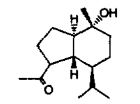

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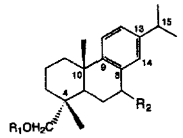
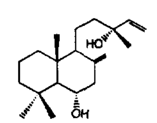


3 R_1=H, R_2=H_2
4 R_1=H, R_2=O
4a R_1=Ac, R_2=O



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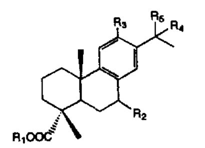
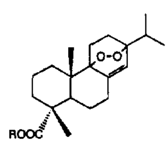
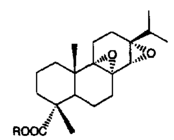


	R_1	R_2	R_3	R_4	R_5
6	H	:O	H	H	Me
6a	Me	:O	H	H	Me
9	H	H	H	H	Me
9a	Me	H	H	H	Me
10	H	α-OH	H	H	Me
10a	Me	α-OH	H	H	Me
12	H	H	H	OH	Me
12a	Me	H	H	OH	Me
13	H	H	H	-	$=CH_2$
13a	Me	H	H	-	$=CH_2$
14	H	H	H	-	:O
14a	Me	H	H	-	:O
15	H	H	OH	OH	Me
15a	Me	H	OH	OH	Me



7 R = H, 9α,13α-epidioxy
7a R = Me, 9α,13α-epidioxy
8 R = H, 9β,13β-epidioxy
8a R = Me, 9β,13β-epidioxy



11 R = H
11a R = Me

roxydehydroabietic acid (**10**) [19], 8α,9α,13α,14α-diepoxyabietan-18-oic acid (**11**) [20] and 15-hydroxydehydroabietic acid (**12**) [18], as methyl esters, **6a**–**12a**. Of these known compounds **2**, **5** and **9a** were identified by direct comparison with their respective authentic samples. Compounds **1**, **3**, **6a**–**8a** and **10a**–**12a** were characterized by the facts that their physical and spectral data were consistent with those already published. The physical and spectral data of the acetate of **4** (**4a**) and **11a** were also in accord with those already published, although the ^{13}C NMR spectrum of **4** has not been reported in the literature and the assignments of signals for C-6, C-8, C-9, C-11 and C-13 in **11a** were inconsistent with those already published [20]. Unambiguous ^{1}H and ^{13}C NMR spectral data of **4** and **11a** obtained from ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, COLOC and NOESY experiments are shown in Tables 1 and 2.

Compound **13a** was assigned the molecular formula $C_{21}H_{28}O_2$ from the HREI mass spectrum. The UV, IR and ^{1}H and ^{13}C NMR spectra (Table 1) showed the presence of two quaternary methyl groups, an isopropenyl group attached to a benzene ring [δ_H 2.12 (3H, *br s*, Me-17), 5.02 (1H, *t*, J = 1.8 Hz, H-16a) and 5.32 (1H, *br s*, H-16b); δ_C 21.7 (*q*, C-17), 111.6 (*t*, C-16) and 138.3 (*s*, C-15)], five methylene groups, a methine group, a methoxycarbonyl group [ν_{max}^{KBr} cm^{-1}: 1710; δ_H 3.66 (3H, *s*); δ_C 52.0 (*q*) and 179.1 (*s*)] and a 1,2,4-trisubstituted benzene ring [λ_{max} 240 and 276 nm; ν_{max}^{KBr} cm^{-1}: 1620, 1595 and 1495; δ_H 7.13 (1H, *d*, J = 1.8 Hz, H-14), 7.20 (1H, *d*, $J_{11,12}$ = 8.4 Hz, H-11) and 7.26 (1H, *dd*, $J_{12,11}$ = 8.4, 1.8 Hz, $J_{12,14}$ = 1.8 Hz, H-12)]. The ^{1}H and ^{13}C NMR signals of **13a** were assigned by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and long range ^{1}H–^{13}C COSY (HETCOR). Except for the presence of an isopropenyl group and the absence of an isopropyl group, the above NMR data were closely similar to those of **9a** [18], suggesting **13a** to be methyl abieta-8,11,13,15-tetraen-18-oate. Together with the EI mass spectrum (see Experimental), the long range ^{1}H–^{13}C COSY data supported this presumption. In the latter experiment (Table 1), **13a** showed cross peaks for signals of H-16 (with C-17) and Me-17 (with C-16, C-15 and C-13), indicative of an isopropenyl group at C-13. A complete proof for the structure was obtained by synthesis. Oxidation of **6a** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane furnished methyl abieta-8,11,13,15-tetraen-18-oate identical in all respects with **13a**. Although **13a** had already been synthesized from methyl dehydroabietate [21–23] and also detected from some methylated pine resins by GC [24] and from the coalified or 'fossilized' products of plant resins by pyrolytic GC-mass spectral analyses in the presence of tetramethylammonium hydroxide [25], this is the first report for the isolation of **13**, as **13a**, from natural sources.

Compound **14a** was assigned the molecular formula $C_{20}H_{26}O_3$ from the HREI mass spectrum. Its UV spectrum exhibited absorption bands similar to those of acetophenone [26] at λ_{max} 240 and 283 nm, while the IR spectrum revealed absorptions for an ester carbonyl and a ketone conjugated with a benzene ring [ν_{max}^{KBr} cm^{-1}: 1735, 1682, 1603, 1546, and 1508]. The ^{1}H and ^{13}C NMR spectra (Table 2) showed signals for two quaternary methyl groups, an acetyl methyl group [δ_H 2.56 (3H, *s*, Me-17); δ_C 26.6 (*q*, C-17) and 198.1 (*s*, C-15)], a 1,2,4-trisubstituted benzene ring [δ_H 7.34 (1H, *d*, $J_{11,12}$ = 8.4 Hz, H-11), 7.64 (1H, *d*, $J_{12,14}$ = 1.8 Hz, H-14) and 7.72 (1H, *dd*, $J_{11,12}$ = 8.4 Hz, $J_{12,14}$ = 1.8 Hz, H-12)] and a methoxycarbonyl group [δ_H 3.68 (3H, *s*); δ_C 52.0 (*q*) and 178.9 (*s*, C-18)], which were closely similar to those observed in **13a**, although the signals of H-14, H-12 and H-11 in the benzene ring were slightly shifted to downfield. The ^{1}H and ^{13}C NMR signals were assigned by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and long range ^{1}H–^{13}C COSY. Together with the carbon–carbon connectivity obtained from the long range ^{1}H–^{13}C COSY experiment (Table 2), the absence of an isopropenyl group and the presence of an acetyl group in **14a** indicated that it must be methyl 16-*nor*-15-oxodehydroabietate. This structure was proved by synthesis. Oxidation of **13a** with OsO_4, followed by $NaIO_4$ furnished the keto-ester identical in all respects with **14a**.

Compound **15a** was assigned the molecular formula $C_{21}H_{30}O_4$ from the HREI mass spectrum. The IR spectrum showed strong absorptions for hydroxyl, an ester carbonyl and an aromatic ring [$\nu_{max}^{CHCl_3}$ cm^{-1}: 3340,

Table 1. ^{1}H and ^{13}C NMR data of **4**, **11a** and **13a** (in $CDCl_3$, TMS = 0)*

	δ_H				δ_C			
H	**4**	**11a**	**13a**	C	**4**	**11a**	**13a**	Long range ^{13}C–^{1}H COSY of **13a** (C to H)
1α	1.54 *m*	1.38 *m*	1.50 *ddd* (12.3, 4.2, 4.2)	1	37.5 *t*	32.7 *t*	37.9 *t*	Me-20
1β	2.31 *ddd* (12.3, 3.0, 3.0)	1.71 *ddd* (12.0, 3.5, 3.5)	2.31 *ddd* (12.3, 3.0, 3.0)					
2	1.75 (2H) *m*	1.59 (2H) *m*	1.72 (2H) *m*	2	18.2 *t*	17.6 *t*	18.6 *t*	H-1, H-3
3α	1.54 *m*	1.74 *m*	1.65 *m*	3	34.8 *t*	36.2 *t*	36.6 *t*	H-1, Me-19
3β	1.38 *ddd* (12.9, 2.4, 2.4)	1.52 *ddd* (12.0, 5.0, 3.0)	1.65 *m*					
4	—	—	—	4	37.7 *s*	47.0 *s*	47.7 *s*	H-2, H-3, H-5 Me-19
5α	2.25 *dd* (10.8, 6.9)	2.35 *dd* (13.0, 3.0)	2.23 *dd* (12.3, 2.1)	5	42.5 *d*	37.6 *d*	44.8 *d*	H-1, H-6, Me-20
6α	2.65 *m*	1.01 *m*	1.40 *m*	6	36.0 *t*	19.2 *t*	21.8 *t*	H-5, H-7
6β	2.65 *m*	1.40 *m*	1.84 *m*					
7α	—	2.15 *ddd* (15.5, 10.5, 9.0)	2.90 *m*	7	200.0 *s*	25.9 *t*	30.1 *t*	H-5, H-6
7β		1.85 *m*	2.90 *m*					
8	—	—	—	8	130.7 *s*	58.5 *s*	134.8 *s*	H-6, H-7, H-14
9	—	—	—	9	153.6 *s*	65.3 *s*	148.8 *s*	H-11, H-14, Me-20
10	—	—	—	10	37.5 *s*	36.9 *s*	37.1 *s*	H-1, H-5, Me-20
11	7.28 *d* (8.2)	1.58 *m* 1.83 *m*	7.20 *d* (8.4)	11	123.7 *d*	21.1 *t*	124.1 *d*	H-12
12	7.38 *dd* (8.2, 2.1)	1.62 *m* 1.83 *m*	7.26 *dd* (8.4, 1.8)	12	132.5 *d*	23.1 *t*	123.0 *d*	H-11, H-14
13	—	—	—	13	146.6 *s*	62.6 *s*	143.0 *s*	H-12, H-14, Me-17
14	7.82 *d* (2.1)	1.78 *s*	7.13 *br s*	14	124.9 *d*	57.5 *d*	126.1 *d*	H-12
15	2.90 *sept.* (6.9)	1.60 *sept.* (7.0)	—	15	33.6 *d*	33.5 *d*	138.3 *s*	H-12, H-14, H-16 Me-17
16	1.22 *d* (6.9)	0.93† *d* (7.0)	5.02 *t* (1.5) 5.32 *br s*	16	23.9 *q*	17.7‡ *q*	111.6 *t*	Me-17
17	1.23 *d* (6.9)	0.97† *d* (7.0)	2.12 *br s*	17	23.9 *q*	18.1‡ *q*	21.7 *q*	H-16
18	3.15 *d* (10.8) 3.45 *d* (10.8)	—	—	18	71.0 *t*	178.9 *s*	179.1 *s*	Me-19, –COOMe
19	0.93 *s*	1.16 *s*	1.28 *s*	19	17.2 *q*	16.4 *q*	16.5 *q*	H-3, H-5
20	1.25 *s*	1.02 *s*	1.21 *s*	20	23.8 *q*	16.8 *q*	25.0 *q*	H-1, H-5
–CO_2Me	—	3.64 *s*	3.66 *s*	–CO_2Me	—	51.9 *q*	52.0 *q*	

* ^{1}H NMR: **4**, **13a** at 300 MHz and **11a** at 500 MHz. ^{13}C NMR: **4**, **13a** at 74.5 MHz and **11a** at 125 MHz.

†,‡ Assignments in each column may be interchangeable.

Table 2. ^{1}H and ^{13}C NMR data of **14a** and **15a** (in $CDCl_3$, TMS = 0)*

H	δ_H **14a**	δ_H **15a**	C	δ_C **14a**	δ_C **15a**	Long range ^{13}C–^{1}H COSY of **14a** (C to H)
1α	1.50 *m*	1.47 *m*	1	37.7 *t*	37.8 *t*	H-2, Me-20
1β	2.34 *ddd* (12.0, 3.0)	2.24 *ddd* (12.5, 3.0, 3.0)				
2	1.76 (2H) *m*	1.69 (2H) *m*	2	18.4 *t*	18.5 *t*	H-1, H-3
3α	1.71 *m*	1.64 *m*	3	36.5 *t*	36.6 *t*	H-1, Me-19
3β	1.71 *m*	1.75 *m*				
4	—	—	4	47.6 *s*	47.7 *s*	H-2, H-5, Me-19
5	2.22 *dd* (12.6, 2.3)	2.19 *dd* (12.0, 2.5)	5	44.5 *d*	44.7 *d*	H-1, H-6, Me-20
6α	1.54 *m*	1.38 *m*	6	21.4 *t*	21.8 *t*	H-5, H-7
6β	1.85 *m*	1.79 *m*				
7	2.96 (2H) *m*	2.78 (2H) *m*	7	29.9 *t*	29.3 *t*	H-5, H-6
8	—	—	8	135.4 *s*	125.7 *s*	H-7, H-11
9	—	—	9	154.9 *s*	150.3 *s*	H-14, Me-20
10	—	—	10	37.7 *s*	37.0 *s*	H-1, H-5, Me-20
11	7.34 *d* (8.4)	6.76 *s*	11	124.6 *d*	112.9 *d*	H-12
12	7.72 *dd* (8.4, 1.8)	8.61† (O<u>H</u>)	12	125.8 *d*	153.4 *d*	H-14
13	—	—	13	134.5 *s*	128.6 *s*	H-11, Me-17
14	7.64 *d*	6.72 *s*	14	129.3 *d*	125.7 *d*	H-12
15	—	2.36† (O<u>H</u>)	15	198.1 *s*	75.7 *s*	Me-17
16	—	1.63‡ *s*	16	—	30.2 *q*	—
17	2.56 *s*	1.66‡ *s*	17	26.6 *q*	30.3 *q*	
18	—	—	18	178.9 *s*	178.2 *s*	Me-19, -COO<u>Me</u>
19	1.29 *s*	1.26 *s*	19	16.5 *q*	16.5 *q*	H-3, H-5
20	1.22 *s*	1.20 *s*	20	24.8 *q*	24.8 *q*	H-5
$-CO_2$<u>Me</u>	3.68 *s*	3.66 *s*	$-CO_2$<u>Me</u>	52.0 *q*	51.9 *q*	

* ^{1}H NMR: **14a** at 300 MHz and **15a** at 500 MHz. ^{13}C NMR: **14a** at 74.5 MHz and **15a** at 125 MHz.
† Exchanges with D_2O.
‡ Assignments in column may be interchangeable.

1710, 1615, 1560 and 1495]. The ^{1}H and ^{13}C NMR spectra (Table 2) revealed resonances for two quaternary methyl groups, a hydroxyisopropyl group [δ_H 1.63 and 1.66 (each 3H, *s*, Me-16 and Me-17); δ_C 30.2 and 30.3 (each *s*, C-16 and C-17) and 75.7 (*s*, C-15)], a tetrasubstituted benzene ring [δ_H 6.72 and 6.76 (each 1H, *s*, H-14 and H-11)], a phenolic hydroxyl proton which disappeared on the addition of D_2O [δ_H 8.61 (1H, *br s*, OH-12)] and a methoxycarbonyl group [δ_H 3.66 (3H, *s*); δ_C 51.9 (*q*, CO_2Me) and 178.2 (*s*, C-18)]. Although the signal patterns due to the benzene ring in the ^{1}H and ^{13}C NMR spectra of **15a** were different from those of **12a**, the methyl resonances were closely similar to each other. These ^{1}H and ^{13}C NMR signals were assigned by analysing the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, COLOC and NOESY data. In the COLOC spectrum (Fig. 1), C-8, C-10, C-12 and C-13 correlated with H-11, as well as with C-7, C-8, C-9, C-12 and C-15 with H-14. Furthermore, significant NOE enhancements were observed for the signals of H-11 (with H-1β and Me-20), H-14 (with H-7β, Me-16 and Me-17), H-5α (with H-7α) and the hydroxyl proton on the 12 position (with Me-16 and Me-17) in the NOESY experiment (Fig. 1). Consequently, the phenolic hydroxyl group of **15a** was located at C-12 and the structure was established as methyl 12,15-dihydroxydehydroabietate. Although a considerable number of dehydroabietic acid analogues have hitherto been isolated from various natural resources [27], to the best of our knowledge compounds **14a** and **15a** have not yet been reported in the literature.

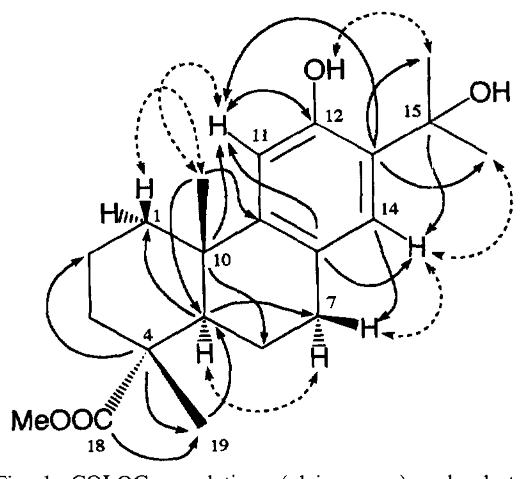


Fig. 1. COLOC correlations (plain arrow) and selected NOESY interactions (dashed arrow) of **15a**

EXPERIMENTAL

General. Mps: uncorr.; Optical rotations: $CHCl_3$, unless otherwise noted; UV: EtOH; IR: $CHCl_3$ and KBr discs; ^1H NMR (500 and 300 MHz) and ^{13}C NMR (125 and 74.5 MHz): $CDCl_3$ with TMS as int. standard; EIMS (probe): 70 eV; CC: Kiesel gel 60 and alumina 90 (70–230 mesh, Merck); TLC and prep. TLC: silica gel HF_{254} and PF_{254} (Merck).

Plant material. The leaves of *L. kaempferi* were collected at about 800 m above sea level on Mt. Daisen, Tottori Prefecture, Japan, on July 29th, 1994. The plant material was identified by Mr M. Ohmachi of National Kurayoshi Forestry Office. A voucher specimen of *L. kaempferi* (94P-LK-01) is deposited on file at the Herbarium of the Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and isolation of compounds. The air dried leaves of *L. kaempferi* (4.6 kg) were extracted with CH_2Cl_2 (10 l × 5) employing an automatic glass percolator and the resulting CH_2Cl_2 soln was evapd *in vacuo* to yield a dark green tarry residue (272.6 g), which was subjected to CC on silica gel (3.6 kg). Elution of the column with $CHCl_3$ afforded four dark green tarry residues, A (5.18 g), B (6.78 g), C (13.76 g) and D (32.96 g), from fr. nos. 10–13, 30–36, 37–40 and 41–50 (each fr: 1 l), respectively. Continuous elution with $CHCl_3$–EtOAc (10:1) yielded a dark green gummy residue E (13.78 g) from fr. nos. 77–94 (each fr.: 1 l). Residue B was rechromatographed on a silica gel (300 g) column to yield residues B-1 (270 mg), B-2 (3.33 g) and B-3 (723 mg) from fr. nos. 3–5, 6–10 and 11–14, eluted with $CHCl_3$ (each fr.: 200 ml), respectively. Continuous elution with $CHCl_3$–EtOAc (10:1 and 5:1) and EtOAc successively afforded residues B-4 (2.8 g, from frs 16–32), B-5 (14 mg, from frs 44–50) and B-6 (115 mg, from frs 76–81). Repeated CC of residue B-1 on Al_2O_3 (50 g) using C_6H_6 furnished dehydroabietinol (**3**), 10 mg, as a colourless oil, $[\alpha]_D^{23}$ +39° (*c* 0.80); EIMS: *m/z* 286 $[M]^+$, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 3050, 1605, 1570, 1510, 1500 and 1460. Repeated CC of residue B-3 with $AgNO_3$–Al_2O_3 (1:9) afforded 7-oxodehydroabietinol (**4**), 8 mg, as a colourless oil, $[\alpha]_D^{23}$ +18° (*c* 0.61), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3005, 1670, 1603, 1490 and 1460, EIMS: *m/z* 300 $[M]^+$, ^1H and ^{13}C NMR: see Table 1; from the frs eluted with *n*-hexane–C_6H_6 (5:1). Usual acetylation of **4** furnished the monoacetate **4a** as a colourless oil, $[\alpha]_D^{23}$ +23° (*c* 0.8), (lit. [14], $[\alpha]_D$ +20°), EIMS: *m/z* 342 $[M]^+$. Physical and spectral data ($[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) of compounds **3** and **4a** were in good agreement with those already published. Residues B-4, B-5 and B-6, showing IR absorption bands for a carboxyl group, were treated with CH_2N_2–Et_2O to yield three corresponding products, B-4a, B-5a and B-6a. Repeated CC of B-4a on $AgNO_3$–Al_2O_3 (1:9) afforded methyl 7-oxodehydroabietate (**6a**), 20 mg, as a colourless oil, $[\alpha]_D^{23}$ +11° (*c* 0.75), (lit. [16], $[\alpha]_D$ +20°), EIMS: *m/z* 328 $[M]^+$, from the fr. eluted with $CHCl_3$–EtOAc (5:1). Physical and spectral data of **6a** were in accordance with those already published. Residue B-5a was purified by prep. TLC (plate: 0.5 mm thick, 20 × 20 cm; solvent: $CHCl_3$–MeOH, 80:1) to give methyl 9α, 13α-epidioxyabiet-8(14)-en-18-oate (**7a**), 13 mg, as a colourless oil, $[\alpha]_D^{23}$ −19° (*c* 0.69) (lit. [17], $[\alpha]_D^{25}$ −19°), EIMS: *m/z* 348 $[M]^+$. Purification of residue B-6a by prep. TLC (plate: 0.5 mm thick, 20 × 20 cm; solvent: $CHCl_3$–MeOH, 80:1) furnished methyl 9β,13β-epidioxyabiet-8(14)-en-18-oate (**8a**), 10 mg, as a colourless oil, $[\alpha]_D^{23}$ −32° (*c* 0.72) (lit. [17], $[\alpha]_D^{25}$ −32°), EIMS: *m/z* 348 $[M]^+$. The structures of compounds **7a** and **8a** were identified by comparing their physical and spectral data (IR, ^1H and ^{13}C NMR and EIMS) with those already published. Rechromatography of residue C on silica gel (300 g) column afforded a gum (3.77 g) from the fr. nos. 15–32 eluted with *n*-hexane–C_6H_6 (1:1, each fr.: 50 ml), which was rechromatographed on Al_2O_3 to furnish (−)-α-cadinol (**1**), 24 mg, as needles, mp 71–72° (MeOH), $[\alpha]_D^{23}$ −37° (*c* 0.23) (lit. [11], mp 73–74°, $[\alpha]_D$ −37°); IR ν_{max}^{KBr} cm^{-1}: 3410, 1620, 1385, 1368, 905 and 879; EIMS: *m/z* 222 $[M]^+$, ^1H NMR: δ 0.77 (3H, *d*, *J* = 6.9 Hz, Me-13), 0.97 (3H, *d*, *J* = 6.9 Hz, Me-14), 1.05 (3H, *s*, Me-15), 1.67 (3H, *br s*, $W_{1/2}$ = 4.3 Hz, Me-11) and 5.50 (1H, *br s*, $W_{1/2}$ = 4.5 Hz, H-5α), eluted with *n*-hexane–C_6H_6 (1:1). The physical and spectral data of **1** were coincident with those already published. Further elution with the same solvent yielded an acidic gum (4.57 g) from frs 60–71. Treatment of the acidic gum with CH_2N_2–Et_2O yielded a residue, which was subjected to CC on Al_2O_3. Elution with *n*-hexane–C_6H_6 (1:1) afforded methyl dehydroabietate (**9a**), 924 mg, as needles. Mp 61–62° (EtOH), $[\alpha]_D^{23}$ +51° (EtOH; *c* 0.4) {lit. [18], mp 59–61°, $[\alpha]_D$ +53° (EtOH)}, EIMS: *m/z* 314 $[M]^+$, from fr. nos. 60–71 eluted with the same solvent of *n*-hexane–C_6H_6 (1:1). The structure of compound **1** was confirmed by the fact that its physical and spectral data were in good agreement with those already published, while **9a** was identified by direct comparison (co-TLC, mmp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) with an authentic specimen prepd from a commercial **9**. Repeated CC of residue D on silica gel (500 g) yielded two acidic frs, D-1 (3.94 g) and D-2 (1.93 g), from the fr. nos. 33–77 and 107–116 eluted with $CHCl_3$–EtOAc (10:1–5:1) and the EtOAc (each fr.: 300 ml), respectively. Individual treatment of D-1 and D-2 with CH_2N_2–Et_2O gave residues D-1a and D-2a. Rechromatography of D-1a on an Al_2O_3 column afforded an additional methyl dehydroabietate (**9a**), 1.08 g, from the fr. eluted with *n*-hexane–C_6H_6 (10:1) and a mixt. of esters exhibiting two spots on TLC plate from the fr. eluted with *n*-hexane–C_6H_6 (5:1). The ester mixt. was sepd by prep. TLC (plate: 0.5 mm thick, 20 × 20 cm; solvent: $CHCl_3$–MeOH, 100:1) to furnish compounds **13a** (30 mg) and **14a** (4 mg). Continuous elution with C_6H_6–$CHCl_3$ (2:1) furnished methyl 7α-hydroxydehydroabietate (**10a**), 7 mg, mp 103–105°, $[\alpha]_D^{23}$ +4° (*c* 0.5) {lit. [19], mp 105–107°, $[\alpha]_D$ +15° (EtOH)}, EIMS: *m/z* 330

$[M]^+$. The physical and spectral data (IR, ^1H and ^{13}C NMR and EIMS) of **10a** were in good agreement with those already published. Repeated CC of D-2a with Al_2O_3 (200 g) furnished methyl 8α,9α,13α,14α-diepoxyabietan-18-oate (**11a**), 27 mg, mp 133–134° (*n*-hexane), $[\alpha]_D^{23}$ +56° (*c* 0.75) {lit. [20], mp 130–132°, $[\alpha]_D$ +22.9° (MeOH)}, EIMS: *m/z* 348 $[M]^+$, ^1H and ^{13}C NMR: see Table 1, and methyl 15-hydroxydehydroabietate (**12a**), 111 mg, mp 78–79° (*n*-hexane), $[\alpha]_D^{23}$ +32° (*c* 0.75) {lit. [18], mp 82–83°, $[\alpha]_D$ +54° (EtOH)}, EIMS: *m/z* 330 $[M]^+$, from the frs eluted with C_6H_6–$CHCl_3$ (2:1) and $CHCl_3$, respectively. The physical and spectral data of **11a** and **12a** were compatible with those already published, respectively, except for the ^{13}C NMR data of **11a** as described above. Further elution with EtOAc provided compound **15a**, 13 mg. Residue E was subjected to silica gel CC and the resulting gummy products eluted with $CHCl_3$–EtOAc (10:1–1:1) and EtOAc were combined. Repeated CC of the resulting gum on Al_2O_3 using C_6H_6–$CHCl_3$ (5:1) successively furnished oplopanone (**2**), 42 mg, mp 94–96° (*n*-hexane–$CHCl_3$), $[\alpha]_D^{23}$ −13° (*c* 0.2) (lit. [12], mp 96–97°, $[\alpha]_D^{23}$ −20°, EIMS: *m/z* 238 $[M]^+$, and larixol (**5**), 68 mg, mp 99–101° (*n*-hexane–$CHCl_3$), $[\alpha]_D^{23}$ +40° (*c* 0.95) (lit. [15], mp 101°, $[\alpha]_D$ +57°), EIMS: *m/z* 306 $[M]^+$. Compound **2** was identified by direct comparison (co-TLC, mmp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) with an authentic sample, while compound **5** was confirmed by comparing its $[\alpha]_D$ and IR, ^1H and ^{13}C NMR and EIMS spectral data with those of an authentic sample.

Methyl abieta-8,11,13,15-tetraen-18-oate (**13a**). Needles, mp 73–74° (MeOH), α_D^{23} +55° (*c* 1.0) (lit. [21], mp 74–75°, $[\alpha]_D$ +64°); HREIMS: *m/z* 312.2079 $[M]^+$ (Calcd for $C_{21}H_{28}O_2$: 312.2088); UV λ_{max} nm (log ε): 276 (3.70) and 240 (3.93); IR ν_{max}^{KBr} cm^{-1}. 3010, 2995, 2925, 2860, 1710 (ester C=O), 1620 (>C=C<), 1595 and 1495 (aromatic ring), 1243 and 1170 (ester C—O); ^1H and ^{13}C NMR: see Table 1; EIMS: *m/z* (rel. int.): 312 $[M]^+$ (25), 297 $[M\text{-}Me]^+$ (14), 237 (100) and 171 (53).

Synthesis of **13a**. Methyl dehydroabietate (**6a**) (135 mg) was added into a soln of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (140 mg) in 1,4-dioxane (10 ml) and the mixt. was refluxed for 5 hr. Evapn of the mixt. *in vacuo* furnished a residue, which was purified through Al_2O_3 CC. Recrystallization from MeOH furnished crystalline methyl abieta-8,11,13,15-tetraen-18-oate (53 mg), which was identified by direct comparison (co-TLC, mmp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) with those of **13a** obtained from the leaves of *L. kaempferi*.

Methyl 16-nor-15-oxodehydroabietate (**14a**). Prisms, mp 84–85° (*n*-hexane–$CHCl_3$), $[\alpha]_D^{23}$ +45° (*c* 0.3); HREIMS: *m/z* 314.1880 $[M]^+$ ($C_{20}H_{26}O_2$ requires 314.1880); UV λ_{max} nm (log ε): 283 (3.96) and 240 (4.02); IR ν_{max}^{KBr} cm^{-1}: 3017, 3003, 2993, 1735 (CO_2Me), 1682 (Ar-C=O), 1603, 1508 and 1406 (aromatic ring), 1250 and 1173 (ester C–O); ^1H and ^{13}C NMR: Table 2; EIMS: *m/z* (rel. int.): 314 $[M]^+$ (34), 299 $[M\text{-}Me]^+$ (14), 239 (100) and 173 (53).

Synthesis of **14a** *from* **13a**. Compound **13a** (50 mg), derived from commercial dehydroabietic acid, was added into a soln of OsO_4 (2 mg) in THF (20 ml) and H_2O (30 ml) under stirring at room temp. After standing for 15 min, $NaIO_4$ (90 mg) was gradually added into the reaction mixt. for 30 min. and the mixt. was further stirred at room temp. for 3 hr. Then the reaction content was filtered and the resulting soln was evapd *in vacuo* to give a residue, which was dissolved in $CHCl_3$ and washed in order with 2 N NaOH and H_2O, and then the $CHCl_3$ layer was dried over Na_2SO_4. Evapn of the solvent *in vacuo* yielded a solid (54 mg). Purification by prep. TLC (solvent: $CHCl_3$–MeOH, 100:1) furnished methyl 16-*nor*-15-oxodehydroabietate (21 mg), which was identified by direct comparison (co-TLC, mmp, $[\alpha]_D$, UV, IR, ^1H and ^{13}C NMR and EIMS) with compound **14a** isolated from the leaves.

Methyl 12,15-dihydroxydehydroabietate (**15a**). Colourless oil, $[\alpha]_D^{23}$ +29° (*c* 1.2); HREIMS: *m/z* 346.2140 $[M]^+$ ($C_{21}H_{30}O_4$ requires 346.2143); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340 (OH), 2970, 1710 (CO_2Me), 1615, 1560, 1495 (aromatic ring), 1243 and 1160 (ester C—O); ^1H and ^{13}C NMR: Table 2; EIMS: *m/z* (rel. int.): 346 $[M]^+$ (3), 328 $[M\text{-}H_2O]^+$ (59), 253 (100) $[M\text{-}H_2O\text{-}HCOOMe]^+$ and 147 (5).

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