Tetrahedron Vol. 49, No. 36, pp. 7903-7915, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Further Acidic Constituents and Neutral Components of *Pinus massoniana* Resin†

H.T.Andrew Cheung,* Toshio Miyase,[1] Mark P. Lenguyen, and Mary A. Smal

Department of Pharmacy, University of Sydney, Sydney, NSW 2006, Australia

(*Received in USA* 24 *March* 1993; *accepted* 9 *June* 1993)

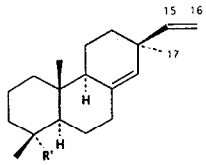
Abstract: The resin of *Pinus massoniana* Lamb. contains, in addition to those isolated in an earlier study, two new diterpene acids, *viz.* 15-hydroxy-7,13-abietadien-12-on-18-oic acid and 8(14)-podocarpen-7,13-dion-18-oic acid, and three new plant products, *viz.* 2β-hydroxy-8,11,13-abietatrien-18-oic acid, 7,13,15-abietatrien-18-oic acid, and 13-hydroxy-8,11,13-podocarpatrien-18-oic acid. The above acids and four others from the resin were isolated as the p-nitrophenyl esters, and the structures of these were established from spectroscopic evidence. Also isolated were three neutral di- and sesquiterpenes.

The resin exuded from the trunk of *Pinus massoniana* Lamb. is a traditional Chinese medication. Following up a report of Liu and his coworkers[2] on antithrombotic and anti-platelet-aggregation actions of the acidic fraction of the resin, we described in an earlier paper[3] the separation of the acidic components into eight diterpene acids: pimaric (**1**), levopimaric (**2**), palustric (**3**) neoabietic (**4**), abietic (**5**) and dehydroabietic (**6**) acids, as well as 7-oxodehydroabietic acid (**7**) and 7α-hydroxydehydroabietic acid (**8**) which are very minor components. Levopimaric (**2**), pimaric (**1**), palustric (**3**) and neoabietic acids (**4**), in approximate order of decreasing potency, were found to inhibit the aggregation of rabbit platelets induced by the agonists platelet activating factor (PAF), adenosine 5'-diphosphate, and calcium ionophore A23187. With induction by the last two agonists, the activity of levopimaric acid (**2**) is higher than that of the cis-unsaturated fatty acid linolenic acid.

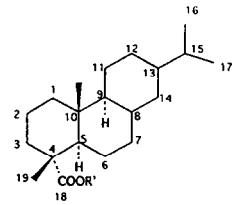
RESULTS AND DISCUSSION

In our previous study, separation of the diterpene acids was *via* the p-nitrophenyl esters. In this work, the most polar fractions of the esters have been separated to yield the p-nitrophenyl esters of six abietane diterpene acids and of three trisnor-diterpene acids. Of these acids, to our knowledge 15-hydroxy-12-keto-abietic acid (15-hydroxy-7,13-abietadien-12-on-18-oic acid) (**14**) and 8(14)-podocarpen-7,13-dion-18-oic acid (**17**) have not been recorded in the literature, while 2β-hydroxydehydroabietic acid (2β-hydroxy-8,11,13-abietatrien-18-oic acid) (**10**), 7,13,15-abietatrien-18-oic acid (**13**), and 13-hydroxy-8,11,13-podocarpatrien-18-oic acid (**15**) are new plant products. The other acids are 15-hydroxydehydroabietic acid (**9**), 7β-hydroxy-

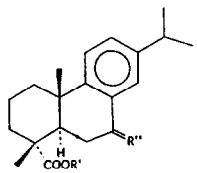
† Dedicated to Sir Derek Barton on the occasion of his 75th birthday.



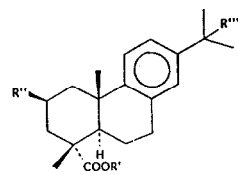
1 R' = COOH
1b R' = CHO



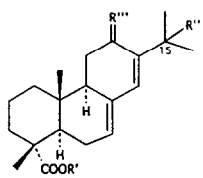
2	$\Delta^{8(14),12}$	R' = H
3	$\Delta^{8,13}$	
4	$\Delta^{8(14),13(15)}$	
5	$\Delta^{7,13}$	
5a	$\Delta^{7,13}$	R' = R



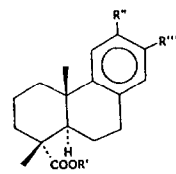
	R'	R"
6	H	H,H
6a	R	H,H
7	H	O
7a	R	O
8	H	α-OH,β-H
8a	R	α-OH,β-H
11	H	α-H,β-OH
11a	R	α-H,β-OH



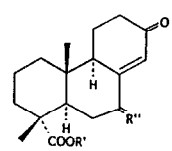
	R'	R"	R"'
9	H	H	OH
9a	R	H	OH
10	H	OH	H
10a	R	OH	H



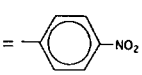
	R'	R"	R"'
12	H	H	α-OH,β-H
12a	R	H	α-OH,β-H
12b	Me	H	α-OAc,β-H
13	H	(Δ^{15})	H,H
13a	R	(Δ^{15})	H,H
13b	Me	(Δ^{15})	H,H
14	H	OH	O
14a	R	OH	O



	R'	R"	R"'
15	H	H	OH
15a	R	H	OH
15b	R	OH	H



	R'	R"
16	H	H,H
16a	R	H,H
16b	Me	H,H
17	H	O
17a	R	O

R = 

dehydroabietic acid 18-oic acid) (**11**), 12α-hydroxyabietic acid (**12**), and 8(14)-podocarpen-13-on-18-oic acid (**16**).

About 4% of *Pinus massoniana* resin consists of a neutral material which was found to also inhibit the PAF-induced aggregation of rabbit platelets. A sample of the neutral material was separated by preparative thin-layer chromatography (TLC), and the inhibitory activity of each fraction was tested. From the equivalent of the most active fraction we have isolated the sesquiterpene hydrocarbon longifolene, while the sesquiterpene β-caryophyllene oxide was obtained from the equivalent of the second most active one. We have also isolated the diterpene 8(14),15-pimaradien-18-al (**1b**).

In the literature, the above known acids tend to have been characterized as the methyl esters. In our work we have therefore provided independent evidence for the identities of all the p-nitrophenyl esters isolated. Specifically, in our own earlier paper[3] the more abundant acids were shown to have the normal but not the *ent* configuration. From biogentic grounds the other acids are likely to have the same absolute configuration. The effects of the neutral and minor acidic components on aggregation of rabbit platelets will be reported elsewhere.

The p-nitrophenyl ester **9a** of 15-hydroxydehydroabietic acid, $C_{26}H_{31}NO_5$ (Table 1), has ^{13}C-NMR data (Table 2) which parallel those of the corresponding ester **6a**[3] of dehydroabietic acid except for C-15 (72.3 ppm) and carbons in its vicinity: C-16/17 (31.8 *vs.* 24.0 ppm, β effect), C-12 (122.2 *vs.* 124.2 ppm, γ effect), and C-14 (125.0 *vs.* 126.9 ppm, γ effect). The ^{1}H NMR signals (Table 3) for the aromatic protons, for H-5, H-1β and, in particular, for the benzylic H-7 protons (δ 2.99 m, $W_{h/2}$ 16 Hz), are nearly identical to those of p-nitrophenyl dehydroabieate (**6a**).[3] 15-Hydroxydehydroabietic acid (**9**) occurs in *Pinus sylvestris*[4] and *P. armandii*,[5] in diseased *P. contorta*[6] and in *Agathis*[7] and *Abies*[8] species; it is also known as a microbial hydroxylation product of dehydroabietic acid.[9] The methyl ester occurs in *Picea abies*.[10]

2β-Hydroxydehydroabietic acid (2β-hydroxy-8,11,13-abietatrien-18-oic acid) (**10**) is a new plant product. Its p-nitrophenyl ester **10a**, $C_{26}H_{31}NO_5$ (Table 1), has ^{13}C NMR data (Table 2) which resemble those of p-nitrophenyl dehydroabietate (**6a**) but with differences at C-2 (67.3 ppm) and C-1/3 (*ca.* 44.5/41.4 *vs.* 37.9 and 36.5 ppm, β effects). The ^{1}H NMR spectrum shows characteristic aromatic and benzylic signals, unusually downfield angular methyl ones, and a methine signal at δ 4.49 (dddd, *J*~4.2 Hz). By decoupling at 500 MHz, the coupling networks C-1 to C-3 and C-5 to C-7 were determined, and hence the presence of an axial 2β-hydroxy group established. The angular methyl signals are assigned by NOE: δ1.53, to H-1β and H-6β (Me at C-10); δ 1.64, to H-3β (Me at C-4). The positions of these methyl signals and the ^{13}C shieldings of the ring-A carbons are affected by the configuration at C-4 and by the presence of a 2β-OH. Such chemical shift differences between the p-nitrophenyl ester of dehydroabietic acid (**6a**) and that of 2β-hydroxydehydroabietic acid (**10a**) as listed in Tables 4 and 5 (in comparison with literature substituent effects) show that these esters have the same configuration at C-4, and confirm the presence of a 2β-OH. 2β-Hydroxydehydroabietic acid (**10**) is previously known as a biodegradation product of dehydroabietic

acid.[15]

The p-nitrophenyl ester **11a** of 7β-hydroxydehydroabietic acid, $C_{26}H_{31}NO_5$ (Table 1), has ^{13}C NMR data (Table 2) which parallel those of its 7-epimer **8a**[3] isolated previously. Below, the data of the two 7-epimers are compared with those of p-nitrophenyl dehydroabietate (**6a**) and with one another. For the 7α-epimer **8a**, there is a -5.0 ppm γ gauche effect of the pseudoaxial 7α-hydroxy group on C-5.[3] In the pseudoequatorial 7β-epimer **11a**, this is replaced by a -1.4 ppm periplanar heteroatom effect.[16] As expected, C-7 and C-6 in the 7α-epimer are more shielded than in the 7β-epimer by about 2.4 ppm, while C-14 is less shielded. 7β-Hydroxydehydroabietic acid (**11**) occurs in diseased *Pinus contorta*[6] and the Douglas fir.[17] It has also been obtained as a microbial product of dehydroabietic acid (**6**).[18]

The p-nitrophenyl ester **12a** of 12α-hydroxyabietic acid, $C_{26}H_{33}NO_5$ (Table 1), has ^{13}C NMR shifts data (Table 2) reminiscent of those of the corresponding ester **5a** of abietic acid,[3] but with differences around C-12, *viz.* C-12 (66.3 *vs.* 27.5 ppm), C-11 (30.7 *vs.* 22.6 ppm, β effect), C-9 (45.1 *vs.* 51.0 ppm, γ gauche effect), and C-15 (32.8 *vs.* 35.0 ppm, γ effect). 12α-Hydroxyabietic acid (**12**) is known to occur in *P. sylvestris*[4] and in an *Abies* species.[8] To compare with the literature data, the p-nitrophenyl ester **11a** was converted to the methyl ester acetate which was found to have ^1H NMR data nearly the same as those recorded[4,8] for methyl 12α-acetoxyabietate (**12b**).

A new abietane diterpene acid isolated as the p-nitrophenyl ester is shown to be 15-hydroxy-12-ketoabietic acid (15-hydroxy-7,13-abietadien-12-on-18-oic acid) (**14**). The presence of an abietane skeleton in the p-nitrophenyl ester **14a**, $C_{26}H_{31}NO_6$ (Table 1), is indicated by the general NMR features (Tables 2,3). The presence of a 12-keto group conjugated to a 7,13-diene system is shown by the untraviolet absorption of the parent acid **14**, $C_{20}H_{28}O_4$ (Table 1), (γ_{max} 290 nm, log ε 3.78), and by NMR signals of the ester for H-7 and H-14 which have the same line shape as, but are more downfield of the corresponding signals given by the 5,13-dienes **5a** and **12a** (H-7, δ 6.17 *vs.* δ 5.38 and 5.58, all with $W_{h/2}$ 11 Hz; H-14, δ 6.89 *vs.* δ 5.77 and 5.87, all singlets). Likewise C-7 (134.4 ppm) and C-14 (142.0 ppm) resonate 11-20 ppm downfield of their counterparts. The presence of a hydroxy group at position 15 on the abietane side-chain is shown by the lowfield positions of the signals of the isopropyl protons (Me, δ 1.44) and carbons (C-15, 71.7 ppm; C-16/17, 29.0 and 29.4 ppm). By ^1H-^1H and ^1H-^{13}C COSY experiments at (for ^1H) 500 MHz, all proton and carbon signals are assigned (Tables 2,3). In particular, the location of a carbonyl at position 12 is shown by the following approximate AMX system: H-11β, δ 2.34, t (J=14.8 Hz); H-11α, δ 2.47, dd (J=5.0,14.8 Hz); and H-9, δ 2.59, d broadened by allylic coupling (J=14.8 Hz). The assignments are supported by long-range ^1H-^{13}C COSY data which *inter alia* show coupling between H-9 and carbons 11 (38.3 ppm) and 8 (133.5 ppm), and between the protons at 11 and carbons 12 (201.8 ppm), 9 (48.4 ppm) and 8. The same data show coupling between C-7 (134.3 ppm) and *inter alia* H-6α and H-6β, and between C-6 (26.7 ppm) and H-5. In turn H-5 is also coupled to carbons 1 (37.7 ppm), 4 (46.7 ppm), 10 (34.9 ppm) and 20 (14.6 ppm).

Another new plant product is 7,13,15-abietatrien-18-oic acid (**13**). Its p-nitrophenyl ester **13a**,

$C_{26}H_{31}NO_4$ (Table 1), has NMR data reminiscent of those of the corresponding ester **5a** of abietic acid,[3] but with differences diagnostic of an additional exomethylene C-15 double-bond in conjugation, *viz.* H-16, singlets at δ 4.93 and 5.05; H-17, methyl singlet at δ 1.93; C-16, 111.5 ppm; C-15, *ca.* 136 ppm. The corresponding methyl ester **13b** was an artifact of *P. armandii*,[5,19] with reported ^1H and (after reassignment) ^{13}C NMR data similar to those of the p-nitrophenyl ester **13a** (Tables 2, 3). The stereochemistry at C-4 and C5 is shown by the shieldings of H-19 and C-19 (see ref. 3 and below).

Turning to the trisnor-diterpenes, 13-hydroxy-8,11,13-podocarpatrien-18-oic acid (**15**) has, to our knowledge, been recorded only once before, as an oxidation product of the sodium salt of levopimaric acid (**2**).[20] Its p-nitrophenyl ester **15a**, $C_{23}H_{25}NO_5$ (Table 1), has NMR data (Tables 2,3) in agreement with the structure. In particular, the ten carbon signals for the sp^3 carbons and the proton signals for H-7 and for H-1β resemble those of p-nitrophenyl dehydroabietate (**6a**)[3] and its 15-hydroxy analogue **9a**, both with an aromatic C-ring. To distinguish from the alternative structure **15b** with an hydroxy group at C-12, the chemical shifts of the aromatic protons of **15a** and **15b** are estimated from those of p-nitrophenyl dehydroabietate (**6a**)[3] using aromatic substituent effects.[21] The estimated chemical shifts for compound **15a** are within 0.02 ppm of those observed, which are H-11, δ 7.14, d (*J*=8.6 Hz); H-12, δ 6.65, dd (*J*=2.7,8.6 Hz); and H-14, δ 6.53, d (*J*=2.7 Hz).

The p-nitrophenyl ester **16a** of 8(14)-podocarpen-13-on-18-oic acid, $C_{23}H_{27}NO_5$ (Table 1), has ^{13}C-NMR chemical shifts data (Table 2) which, for the skeletal nuclei, are nearly identical to those reported for methyl 8(14)-podocarpen-13-on-18-oate (**16b**).[12] 8(14)-Podocarpen-13-on-18-oic acid (**16**) is a feeding deterrent first isolated from *P. banksiana*,[22] and has also been obtained by degradation of abietic acid (**5**).[23]

The 4-epimers of acid **15** and of acid **16** are known, having been obtained recently by degradation of methyl 4-epi-dehydroabietate.[12] However acids **15** and **16** from *P. massoniana* have the same configuration at C-4 as the other acids from the same source, since all the p-nitrophenyl esters are characterized by upfield 4-methyl (C-19) carbon signals due to γ gauche interactions of this axial methyl carbon with carbons 2 and 6.

8(14)-Podocarpen-7,13-dion-18-oic acid (**17**) is a new trisnor-diterpene acid. The p-nitrophenyl ester **17a**, $C_{23}H_{25}NO_6$ (Table 1), is characterized by a very downfield vinyl ^1H NMR signal at δ 6.71, dd (*J*=1.1,3.4 Hz) suggestive of a cisoid enone. Its ^{13}C NMR spectrum (Table 2) resembles that of the enone ester **16a**, but showing an additional signal near 199 ppm for a keto carbonyl. Based on a structure with an additional 7-carbonyl, C-8 is more deshielded by *ca.* 13 ppm, while carbons 5 and 14 (γ to this carbonyl oxygen) are shielded by *ca.* 4 ppm. By ^1H-^{13}C COSY, NOE and ^1H-^1H decoupling experiments, essentially all the proton and carbon signals of of the enone **16a** and the endione **17a** are assigned (Tables 2,3). The 7-carbonyl in the endione **17a** causes deshielding of H-5 and H-9 of *ca.* 0.35 ppm. In comparison, the 7-CO in the C-aromatic compound **7a** deshields H-5 by *ca.* 0.6 ppm.[3]

Turning to the neutral constituents, 8(14),15-pimaradien-18-al (**1b**) has ^1H NMR data (Table 3)

showing striking resemblances to those of pimaric acid (**1**)[3] but with a CHO signal at δ 9.24 replacing that of COOH, and with differences near position 4. Likewise the ^{13}C NMR data (Table 2) parallel those of pimaric acid[3] but with a CHO signal (δ 206.4 ppm) replacing that of COOH, and with shift differences for carbons 3 - 5. The configuration at position 13 is shown by the chemical shift value of the 13-Me carbon (C-17).[24] Recently a GC-MS study of *P. massoniana* resin was carried out, and the 13-epimer of aldehyde **1b** was claimed to be one of the constituents.[25]

The ^1H and ^{13}C NMR spectra of two other neutral constituents isolated have superficial similarities in showing the presence of three angular methyl groups, a $>C=CH_2$ group, and the same number of methyl, methylene, methine and quaternary carbons. The molecular weights are 204 and 220 respectively (see Experimental), leading to the formulae $C_{15}H_{24}$ and $C_{15}H_{24}O$. These constituents have been identified as longifolene and β-caryophyllene oxide. For the former, the methyl and vinyl proton signals obeserved are essentially the same as those reported for this sesquiterpene in other solvents,[26,27] while the ^{13}C NMR data are nearly identical to those in the literature.[27] Longifolene is known to occur in *Pinus* resins.[28,29]

The presence of an epoxide in β-caryophyllene oxide from *P. massoniana* is indicated by the presence of unusually upfield oxygen-bearing quaternary and methine carbon signals at 60.0 and 63.9 ppm respectively, and has been confirmed by treatment with $BF_3.Et_2O$. The ^{13}C NMR data are essentially identical to those in the literature.[30] To our knowledge, β-caryophyllene oxide has not been found in *Pinus* species, although the occurrence of β-caryophyllene is known.[29,31]

Table 1. Molecular Formulae, CH_4CI^a and (*in italics*) FAB Mass Spectral Data

	Formula	$MH^+/M^{+\cdot}$	MH^+-H_2O	$MH-HCOOC_6H_4NO_2$ or $M-OC_6H_4NO_2$	MH^+ of $HOC_6H_4NO_2$	Other ions
9a	$C_{26}H_{31}NO_5$[b]	438 (10)	420 (100)	271 (10)		
9	$C_{20}H_{28}O_3$[b]	317 (50)	299 (100)	-	-	135 (65)
10a	$C_{26}H_{31}NO_5$[b]	438 (40)	420 (30)	271 (100), 253[c] (30)	140 (35)	135 (100)
11a	$C_{26}H_{31}NO_5$[b]	438 (10)	420 (100)	271 (10), 253[c] (15)		
12a	$C_{26}H_{33}NO_5$[b]	440 (15)	422 (100)	273 (20)		
13a	$C_{26}H_{31}NO_4$[d]	422 (100)		255 (40)	140 (20)	
		421		*255*		*149*
14a	$C_{26}H_{31}NO_6$[d]	454 (10)	436 (100)	287 (10), 269[c] (10)	140 (15)	
14	$C_{20}H_{28}O_4$[d]	333 (20)	315 (100)	-	-	
15a	$C_{23}H_{25}NO_5$[b]	396 (30)		229 (60)	140 (100)	
16a	$C_{23}H_{27}NO_5$[b]	398 (100)		231 (25)	140 (20)	
17a	$C_{23}H_{25}NO_6$[b]	*412*		*245*		*307, 289, 219*

[a] Relative abundances given in brackets; $(M+C_2H_5)^+$ and $(M+C_3H_5)^+$ ions also observed. [b] Based on CH_4CI or FAB MS.
[c] With additional loss of H_2O. [d] Based on mass matching on EI MS and/or elemental analysis, and on CH_4CI or FAB MS.

Table 2. 68-126 MHz ^{13}CNMR Data with Chemical Shifts Referenced to the Solvent $CDCl_3$ (77.1 ppm)

Carbon	6a[3]	9a	10a	8a[3]	11a	5a[3]	12a[c]	14a[c]	13a[c]	13b[d]	1b	15a	16a[c]	17a[c]
1	37.9	38.0	44.3[b]	37.7	38.0	38.2	38.0	37.7	38.3	38.3	38.8	38.2	38.3	37.7
2	18.5	18.6	67.3	18.6	18.5	18.1	18.1	17.9	18.2	18.1	19.2	18.7	18.1	17.7
3	36.5	36.5	41.5	35.9	36.5	37.1	37.1	37.1	37.2	37.1	32.6	36.6	36.9*	36.9
4	48.3	48.3	47.5	47.9	48.0	47.3	47.2	46.7	47.3	46.6	49.9	48.3	47.9	46.8
5	44.9	44.9	44.6[b]	39.9	43.5	45.2	43.7	43.5	45.1	45.0	46.8	45.1	48.2	44.1
6	22.1	22.1	21.8	30.9	33.2	25.9	26.2	26.7	26.4*	26.0*	24.8	22.1	24.5	38.8
7	30.1	30.1	30.2	68.2	70.7	120.0	123.6	134.3	123.9	124.7	35.4*	30.2	35.3	198.4*
8	134.4	134.3	133.9	136.0	137.3	135.8	134.5	133.5	136.0**	136.0	137.6	136.2	163.9	151.4
9	146.0*	146.2	147.0*	146.4*	146.3*	51.0	45.1	48.4	50.5	50.3	51.3	141.6	51.8	51.7
10	37.0	37.2	36.9	37.5	37.8	34.7	34.3	34.9	34.8	34.5	37.4	36.9	38.5	36.0
11	124.2	124.2	124.4**	124.4	124.2	22.6	30.7	38.3	22.3	22.1	19.2	125.6	20.6	23.0
12	124.2	122.2	124.8**	126.9	125.3**	27.5	66.3	201.8	26.3*	26.2*	35.7*	113.3	36.8*	38.0
13	146.6*	147.4	146.1*	147.0*	146.8*	145.7	144.3	140.1	143.0	143.2	a	153.2	199.5	199.7*
14	126.9	125.0	127.0	127.9	126.1**	122.3	125.4	142.0	126.7	127.0	128.7	115.0	126.5	130.7
15	33.5	72.3	33.6	33.6	33.9	35.0	32.8	71.7	136.7**	135.8	147.1	-	-	-
16, 17	24.0	31.8	24.1	23.9	24.1	20.9	21.9	29.0	111.5	111.2	112.9	-	-	-
	24.0	31.8	24.1	24.0	24.2	21.4	22.5	29.4	20.7	20.5	29.6	-	-	-
18	176.3	176.2	176.2	176.1	175.8	176.2	176.0	175.7	176.1	179.0	206.4	176.2	176.1	175.2
19	16.6	16.8	18.8	16.7	16.8	17.2	17.2	17.2	17.3	17.0	17.5	16.8	17.4	16.8
20	25.2	25.2	28.1	24.3	25.7	14.1	14.6	14.7	14.3	14.0	14.9	25.4	15.8	15.1
1'	156.1	156.0	156.1	156.2	155.9	156.0	156.0	155.7	156.0	-	-	156.0	155.8	155.6
2', 6'	122.4	122.4	122.4	122.9	122.4	122.5	122.4	122.4	122.5	-	-	122.4	122.4	122.5
3', 5'	125.2	125.2	125.3	125.2	125.2	125.2	125.1	125.2	125.2	-	-	125.2	125.2	125.4
4'	145.2	145.2	145.4	145.2	145.3	145.2	145.2	145.3	145.3	-	-	145.2	145.3	145.6

*, **Assignments within a vertical column may be reversed.

[a] Signal for this quaternary carbon not observed.

[b] These signals may be interchanged since the sample (1.5 mg) was not adequate for experiments to distinguish between CH and CH_2, nor for ^{1}H-^{13}C COSY.

[c] Assignments supported by ^{1}H-^{13}C COSY.

[d] Data of ref. 5 (with no reference standard quoted) reassigned by us.

Table 3. ^{1}H NMR Data[a] *(to be continued)*

	H-19 (4-Me)	H-20 (10-Me)	H-16/17 (15-Me or = CH_2)	H-15	H-14	H-12	H-11	H-9	H-7	H-6	H-5	H-1β	H-1α	H-2	H-3α	H-3β
9	1.29	1.22	1.56	-	7.16 m	7.23 m	7.26 m		2.92 m[b] ($W_{h/2}$ 14)		2.24 dd (2.1,12.4)	2.32 bd (13.0)				
9a	1.43	1.28	1.57	-	7.19m	7.26 m	7.27 m		2.99 m[b] ($W_{h/2}$ 16)		2.42 dd (2.1,12.4)	2.39 bd (13.0)				
10[c,e,f]	1.64	1.53	1.23 d (6.9)	2.83 sep (7.0)	6.91 d (1.8)	7.04 dd (1.8,8.2)	7.20 d (8.2)		2.96 m[b] ($W_{h/2}$ 16)	1.68m(α) 2.05 m (β)	2.47 dd (2.1,12.5)	2.54 ddd (1.3,3.8, 14.1)	1.99 dd (4.4,14.2)	4.49 dddd (4.2)	2.27dd (4.0, 14.0)	2.1 ddd (1.4,4.5, 3 14.1)
11a	1.44	1.34	1.24 d (7.0)	2.89 sep (7.0)	7.39 bs	7.13 dd (1.9,8.3)[d]	*ca.* 7.2 d (*ca.* 8)[d]		4.93 dd (7.2,9.6)		2.43 dd (2.7,11.7)[d]	2.36 bd (13.0)				
12a[g]	1.41	0.88	1.09 d (6.9) 1.11 d (6.9)	2.42 sep (6.9)	5.87	4.31 t (2.6)	1.8-2.0 *ca.* 1.3	2.21 dt (12.1,3.1)	5.58 m ($W_{h/2}$ 11)		*ca.* 2.35	1.8-2.0	*ca.* 1.3	1.69 m[b] ($W_{h/2}$8)	1.8-2.0	1.8-2.0
13a[e,g]	1.41	0.89	1.93 4.93, 5.05	-	6.17	*ca.* 2.2 (α) 2.54 dddd (2.2,2.2, 2.2,16.5)(β)	*ca.* 1.9 *ca.* 1.3	*ca.* 2.0	5.61 m ($W_{h/2}$ 10)	*ca.* 2.0 2.25 m[b]	2.25 m[b]	*ca.* 1.95	*ca.* 1.3	1.67 m ($W_{h/2}$9)[b]	*ca.* 2.0	1.85 dtd (1.7,3.2, 14.5)
14a[c,e,g,h]	1.44	0.94	1.42	-	6.89	-	2.47 dd (5.0,14.8) (α) 2.34 t (14.8)(β)	2.59 bd (14.8)	6.17 m ($W_{h/2}$ 11)	2.14 bd (18.6)[d](α) 2.31 m ($W_{h/2}$36) (β)	2.23 dd (4.0,11.9)[d]	1.85 bd (13.2)	1.24 m ($W_{h/2}$ 32)	1.70 m ($W_{h/2}$18)[b]	1.92 m[b]	

[a] Unless otherwise stated, chemical shifts in δ (with J and $W_{h/2}$ in Hz) with $SiMe_4$ as internal standard in $CDCl_3$ solvent measured at 300 or 270 MHz. Signals are singlets (s) unless otherwise stated; d, t, sep, m and b refer to doublet, triplet, septet, multiplet and broad respectively. Those incompletely described are partly masked by other signals. The p-nitrophenyl esters also give AA'XX' signals at δ 7.19-7.27 and 8.24-8.30.

[b] Two protons; non-first-order. [c] 500 MHz data.

[d] Observed splittings; not necessarily coupling constants.

Table 3. ^{1}H NMR Data[a] *(concluded)*

	H-19 (4-Me)	H-20 (10-Me)	H-16/17 (15-Me or = CH_2)	H-15	H-14	H-12	H-11	H-9	H-7	H-6	H-5	H-1β	H-1α	H-2	H-3α	H-3β
15a	1.42	1.25	-	-	6.53 d (2.7)	6.65 dd (2.7,8.6)	7.14 d (8.6)		2.92 m[b] ($W_{h/2}$ 16)		2.38 dd (2.1,12.4)	2.37 bd				
16a[c,e,g]	1.39	0.91	-	-	5.91 t (1.9)	*ca.* 2.2 (α) 2.43 btd (16.2,4.2) (β)	2.05 m ($W_{h/2}$22) (α) *ca.* 1.8(β)	2.26 dd (5.4,14.0)	2.41 tdt (1.8,6.8, *ca.*15)(α) 2.57 ddd (1.8,4.9, 15.6) (β)	1.52 tdd (2.2,7.0 13.3)(α) *ca.*1.75(β)	2.22 dd (2.9,12.6)	1.86 dt (12.8,3.1)	1.28 td (12.5,5.0)	*ca.* 1.7 m[b]	*ca.* 1.9m[b]	
17a[c,e,f,g,i]	1.45	1.00	-	-	6.71 dd (1.1,3.4)[j]	2.36 ddd (4.9,15.3, 16.2) (α) 2.63 b dt (*ca.* 17, *ca.* 3) (β)	2.22 m(α) ($W_{h/2}$27) 1.83 q of d (4.2) (β)	2.58 d of t (3.6)	-	*ca.* 2.6 m[b]	*ca.* 2.6 m	1.95 bdd (3.3,13.3)	1.35 td (13.3,4.3)	*ca.* 1.8 (α) 1.72 qt (13.3,3.3) (β)	1.93 td (13.3, 4.2)	2.00 dm (13.3)
17a[c,e,f,i] (C_6D_6)	1.07	0.37	-	-	6.95 dd (0.8,3.0)[j]	1.83 ddd (4.9,15.0, 16.5) (α) 2.30 bdt (16.5,*ca.*3) (β)	1.34 m ($W_{h/2}$22) (α) 1.09 q of d (4.2) (β)	1.54 dt (11.3,3.6)	-	2.47 X of ABX (≤3.1, ≥16.5) (α)	2.23,2.25 AB of ABX (with H-6β)	*ca.* 1.2	0.59 td (13.3,4.3)	*ca.* 1.25 (α) *ca.* 1.2 (β)	*ca.* 1.25	*ca.* 1.2
1b[k]	1.09	0.80	1.00		5.16 m ($W_{h/2}$9)				2.10dddd (α)[k] 2.28ddd (β)[k]							

[e] Assignments supported by ^{1}H-^{1}H decoupling or ^{1}H-^{1}H COSY experiment.
[f] Assignments supported by NOE experiment. [g] Assignments supported by ^{1}H-^{13}C COSY experiment.
[h] Assignments supported by ^{1}H-^{13}C long-range COSY experiment.
[i] 35°C. [j] $J_{12\beta,14}$ and $J_{9,14}$ respectively.
[k] Assignment of H-7 based on our earlier work,[3] observed splittings for H-7α being 1.9, 1.9, 5.6, 12.8, 14.2 Hz, and for H-7β being 2.0, 4.8, 14.2 Hz. Other signals are: δ 9.24 (H-18); 5.71, dd (*J*=10.4,17.2 Hz, H-15); 4.96, dd (*J*=1.9,10.4 Hz, H-16Z); 4.91, dd (*J*=1.9,17.2 Hz, H-16E).

Table 4. ^{13}C NMR Substituent Effects

	Observed δ(**10a**)-δ(**6a**)	Effect of 2β-OH in steroids (androstanes)[11]	Effect of 4-epimerization of **16b**[12]	Effect of 2β-OH with 4-epimerization
C-1	6.4	6.5	-0.2	6.3
C-2	48.8	45.8	1.7	47.5
C-3	5.0	7.0	2.8	9.8
C-4	-0.8[a]	-5.3[a]	-2.9	-8.2
C-5	-0.3	0.3	7.1	7.4
C-6	-0.3	-0.3	-0.2	-0.5
C-7	0.1	-0.1	1.5	1.4
C-8	-0.5	-0.6	0.4	-0.2
C-9	0.10-1.0	0.8	-0.9	-0.1
C-10	-0.1	-0.3	1.8	1.5
10-Me	2.9	2.5	-1.1	1.4

[a]The shielding effect of 2β-OH on C-4 (γ gauche effect) as observed in androstanes is not expected for compound **10a** wherein C-4 is quaternary.

Table 5. Substituent Effects on ^{1}H Chemical Shifts of Methyl Groups

	Observed δ(**10a**)-δ(**6a**)	Effect of 2β-OH in triterpenes[13]	Effect of 2β-OH in steroids[14]
4-Me	0.23	0.22	-
10-Me	0.27	0.32	0.25

EXPERIMENTAL

General Procedures

NMR spectra were measured for $CDCl_3$ solutions on a Varian Gemini spectrometer (300 MHz for ^{1}H, 75.4 MHz for ^{13}C) or on JEOL GSX spectrometers (500 MHz for ^{1}H, 125.7 MHz for ^{13}C; 270 MHz for ^{1}H, 67.8 MHz for ^{13}C), using $SiMe_4$ as an internal standard for ^{1}H, and referenced to $\delta(CDCl_3) = 77.1$ ppm for ^{13}C. ^{1}H-^{13}C COSY ($^{1}J_{CH}$) and long-range ^{1}H-^{13}C COSY ($^{2,3}J_{CH}$) experiments were performed using standard JEOL programs (J=140 Hz and J=10 Hz respectively). Nuclear Overhauser effect (NOE) measurements were carried out at 35°C. Chemical ionization mass spectrometry (CI MS) was performed with methane or with ammonia as reactant gas using a Finnigan-Mat TSQ-46 quadrapole mass spectrometer. Fast atom bombardment spectrometry (FAB MS) was carried out on a JEOL JMS-SX102 mass spectrometer using m-nitrobenzyl alcohol as matrix. Electron impact (EI) mass matching was performed on an upgraded Kratos MS-9 mass spectrometer. High pressure liquid chromatography (HPLC) was carried out using either the JASCO System 800, or a Gilson 302 pump equiped with a Rheodyne 7125 injector and JASCO UVIDEC-100-III ultraviolet detector, with monitoring at 280 and 205 nm for MeOH and CH_3CN solutions respectively. Reverse phase HPLC columns used were: for analytical work, Altex Ultrasphere 5μ ODS (4.6 mm i.d. x 150 mm), and for preparative work, Yamamura YMC D-ODS-7 or Develosil ODS-10 (both 20 mm i.d. x 250 mm). For preparative HPLC, samples (*ca.* 20 mg each) were introduced in 300 μl of CH_3CN,

and the flow rate was 5 ml min^{-1}. Low pressure liquid chromatography was carried out either i) under gravity using long columns with height : diameter of about 5:1 for 100-200 mesh silica gel (Ajax), or of about 2:1 for TLC-grade silica (Merck, Kiesegel H); or ii) with suction using short columns of TLC-grade silica of height : diameter of about 2:3.[32] Petroleum used is of boiling range 65-70°C.

Separation of the neutral materials and isolation of the p-nitrophenyl esters of the more polar acids

Pinus massoniana Lamb. resin (100 g) was separated into acidic and neutral materials as described previously,[3] except that extraction of the Et_2O solution (400 ml) by 2M NaOH (150 ml) was repeated until no turbidity was observed upon acidification (4 extractions), and that the resultant Et_2O phase was washed with saturated aqueous NaCl until neutral (6 times).[33] The neutral material constituted 3.8% by weight of the resin.

In our earlier work,[3] the acid components of the resin were converted to the p-nitrophenyl esters, and a "very polar fraction" (1.44 g from 32 g of resin) was separated. This is composed of fraction A (less polar) (0.40 g) and fraction B (more polar) (1.04 g) which, in the present work, were processed separately. Part of fraction A (0.30 g) was chromatographed over 100-200 mesh silica gel (20 g) with elution by $CHCl_3$, followed by rechromatography of the more polar material (0.30 g) under gravity over TLC-grade silica (23 g) with elution by $CHCl_3$ - petroleum (3:7). From the later fractions were obtained, in order of elution, p-nitrophenyl 7β-hydroxy-8,11,13-abietatrien-18-oate (**11a**) (5 mg) and p-nitrophenyl 13-hydroxy-8,11,13-podocarpatrien-18-oate (**15a**) (5 mg). The first major fraction (75 mg) was rechromatographed twice over TLC-grade silica (26 g, then 15 g) with elution by $CHCl_3$ - petroleum (1:9 to 1:4) to give p-nitrophenyl 12α-hydroxy-7,13-abietadien-18-oate (**12a**) (66 mg); final purification was effected by reverse phase preparative HPLC with MeOH - H_2O (85:15) as mobile phase. The second major fraction (43 mg) was separated as above by HPLC yielding p-nitrophenyl 15-hydroxy-8,11,13-abietatrien-18-oate (**9a**) (22 mg). The remainder of fraction A (0.10 g in 20 mg portions) was directly separated by HPLC as before into, in order of elution, ester **9a** (22 mg) and ester **12a** (23 mg).

Part of fraction B (0.22 g) was similarly separated by HPLC but with elution by MeOH - H_2O (80:20) to give as the major fraction a further amount of ester **9a** (35 mg). An earlier HPLC fraction (17 mg) was further chromatographed this time with MeOH - H_2O (70:30) as mobile phase to give, in order of elution, p-nitrophenyl 8(14)-podocarpen-13-on-18-oate (**16a**) (3 mg), and p-nitrophenyl 15-hydroxy-7,13-abietadien-12-on-18-oate (**14a**) (11 mg), *m/z* (CH_4CI) see Table 1; (EI) 438.191 (12%, M-CH_3) ($C_{25}H_{28}NO_6$ requires 438.193), 435.208 (100%, M-H_2O) ($C_{26}H_{29}NO_5$ requires 435.205) (Found, C, 68.2,; H, 7.0; N, 3.1%. $C_{26}H_{31}NO_6$,¼H_2O requires C, 68.2; H, 6.9; N, 3.1%). Another part of fraction B (0.74 g) was separated by HPLC using linear gradient elution with MeOH - H_2O (70:30 to 90:10) to give, in order of elution, p-nitrophenyl 8(14)-podacarpen-7,13-dion-18-oate (**17a**) (11 mg), ester **16a** (20 mg), ester **14a** (38 mg) and ester **9a** (74 mg). A later fraction (80 mg) was rechromatographed isocratically using CH_3CN - H_2O (80:20) to give, in order of elution, ester **9a** (4 mg), p-nitrophenyl 2β-hydroxy-8,11,13-abietatrien-18-oate (**10a**) (1.5

mg), ester **11a** (11 mg), p-nitrophenyl 7,13,15-abietatrien-18-oate (**13a**) (19 mg) with *m/z* (EI) 421.222 (8%, $M^{+\cdot}$) ($C_{26}H_{31}NO_4$ requires 421.225), and ester **12a** (6 mg). Rechromatography of ester **17a** was carried out using CH_3CN - H_2O (60:40) to give pure ester **17a** (3.5 mg).

Hydrolysis to diterpene and trisnor-diterpene acids

Some of the isolated p-nitrophenyl esters were hydrolysed by treatment of an acetone solution (3 volumes) with 2M aqueous KOH at room temperature for 2 h, and worked up as described previously.[3] The acids obtained are relatively polar and the previous method of washing with $NaHCO_3$ to remove p-nitrophenol was not suitable, and preparative TLC over EtOAc - hexane (4:6) was used.

From ester **9a** (22 mg) was obtained, after recrystallization from EtOH - H_2O (1:1), 15-hydroxydehydroabietic acid (**9**) (6 mg), mp 185-187°C. From ester **14a** (11 mg) was obtained, after crystallization from EtOAc - hexane, 15-hydroxy-7,13-abietadien-12-on-18-oic acid (**14**) (5 mg), mp 175-180°C, λ_{max} (MeOH) 290 nm (log ε 3.78), *m/z* (CH_4CI) see Table 1; (EI) 317.177 (40%, M-CH_3) ($C_{19}H_{25}O_4$ requires 317.175), 314.186, (100% M-H_2O) ($C_{20}H_{26}O_3$ requires 314.188).

Ester **12a** (11 mg) in acetone (1 ml) was hydrolysed as above but using 2M aqueous NaOH (0.5 ml). Acid **12** obtained on work-up was treated with an excess of CH_2N_2 in Et_2O at room temperature for 1 h. The methyl ester obtained was treated with Ac_2O - pyridine (1:1, 0.3 ml) at room temperature overnight to give on evaporation methyl 12α-acetoxyabietate (2 mg) with ^1H NMR data essentially identical to those in the literature.[4,8]

Isolation of constituents from the neutral material

The neutral material (0.53 g) in the minimum volume of petroleum was loaded onto a short column of TLC grade silica (10 g) and was chromatographed under suction, 20 ml fractions being collected. From fractions 1-4 eluted with petroleum was obtained longifolene (74 mg), *m/z* (NH_3 and CH_4CI) 205 (100%, MH^+), with ^{13}C NMR data essentially identical to those in the literature.[27] Fractions 5-10 (containing 110 mg) eluted with CH_2Cl_2, and fractions 11-13 (160 mg) eluted with EtOAc were combined and similarly rechromatographed but with elution by CH_2Cl_2 - petroleum (1:3, 1:1, 3:1; 100 ml each) followed by CH_2Cl_2 (180 ml). The major component in fractions 4-8 (20 ml each) containing 18 mg was purified by HPLC using CH_3CN - H_2O (85:15) to give 8(14),15-pimaradien-18-al (**1b**) (3.5 mg), *m/z* (CH_4CI) 287 (100%, MH^+). In another separation, the neutral material (0.52 g) was similarly subjected to vacuum liquid chromatography under suction. Fraction 2 (14 mg) eluted with the loading solvent hexane was further separated on a TLC plate (Merck, Kieselgel $60F_{254}$, 0.25 x 200 x 100 mm height) impregnated[3] with $AgNO_3$, with elution by hexane to yield longifolene (1 mg). Fraction 7 (5 mg) eluted with CH_2Cl_2, and fraction 15 (11 mg) eluted with Et_2O - hexane (1:9) were individually rechromatographed on TLC plates (Kieselgel 60H) to yield respectively 8(14),15-pimaradien-18-al (**1b**) (3 mg), and β-caryophyllene oxide (2 mg), *m/z* (NH_3CI) 221 (45%, MH^+), 203 (75%, MH-H_2O), with ^{13}C NMR data essentially identical to those in the literature.[30]

REFERENCES AND NOTES

1. On leave of absence from the School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan.
2. Liu, T.; Gao, C.; Feng, L. *J. Trad. Chinese Med.* **1985**, *5*, 115-118.
3. Cheung, H.T.A.; Fu, S.-L; Smal, M.A. *Arzneim. Forsch. Drug Res.* **1993**, in press.
4. Lisina, A.I.; Svirskaya, N.M.; Pentegova, V.A. *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* **1974**, 142-143. Roshchin, V.I.; Kolodynskaya, L.A.; Raldugin, V.A.; Pentegova, V.A. *Khim. Prir. Soed.* **1985**, 345-351. Puzanova, V. Yu; Schmidt, E.N.; Radbil, B.A.; Pentegova, V.A. *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* **1986**, 129-132.
5. Fang, J.; Lang, C.; Chen, W.; Cheng, Y. *Phytochem.* **1991**, *30*, 2793-2795.
6. Ayer, W.A.; Migaj, B.S. *Can. J. Bot.* **1989**, *67*, 1426-1428.
7. Carman, R.M.; Marty, R.A. *Aust. J. Chem.* **1970**, *23*, 1457-1464.
8. Barrero, A.F.; Sanchez, J.F; Alvarez-Manzaneda, R.E.J.; Dorado, M.M.; Haidour, A. *Phytochem.* **1990**, *29*, 593-597.
9. Ekman, R.; Sjöholm, R. *Acta Chem. Scand.* **1979**, *33*, 76-78.
10. Norin, T.; Winell, B. *Acta Chem. Scand.* **1972**, *26*, 2289-2296.
11. Blunt, J.W.; Stothers, J.B. *Org. Magn. Reson.* **1977**, *9*, 439-464.
12. Abad, A.; Arnó, M.; Peiró M.; Zaragoza, R.J. *Tetrahedron* **1991**, *47*, 3829-3844.
13. Cheung, H.T.; Williamson, D.G. *Tetrahedron* **1969**, *25*, 119-128.
14. Bhacca, N.S.; Williams, D.H. *Application of NMR Spectroscopy in Organic Chemistry, Illustrations from the Steroid Field*; Prentice-Hall: San Francisco. 1964.
15. Kutney, J.P.; Singh, M.; Hewitt, G.; Salisbury, P.J.; Worth, B.R.; Servizi, J.A.; Martens, D.W.; Gordon, R.W. *Can. J. Chem.* **1981**, *59*, 2334-2341.
16. Eliel, E.L.; Bailey, W.F.; Kopp, L.D.; Willer, R.L.; Grant, D.M.; Bertrand, R.; Christensen, K. A.; Dalling, D.K.; Duch, M.W.; Wenkert, E.; Schell, F.M.; Cochran, D.W. *J. Am. Chem. Soc.* **1975**, *97*, 322-330.
17. Rogers, I.; Mahood, H. In *Advances in The Identification and Analysis of Organic Pollutants in Water*; Keith, L.H. Ed.; Ann Arbor Science, p. 1097-1113.
18. Nakashima, R.; Nishii, S.; Ishida, T.; Yamamoto, Y. *Yukagaku* **1990**, *39*, 191-195.
19. In ref. 5, the C-4 configuration drawn for the methyl ester is in error.
20. Herz, W.; Ligon, R.C.; Turner, J.A.; Blount, J.F. *J. Org. Chem.* **1977**, *42*, 1885-1895.
21. Jackman, L.M.; Sternhell, S. *Applications of NMR Spectroscopy in Organic Chemistry*; Pergamon Press: Oxford. 1969; p. 202. Craik, D. *Ann. Rep. NMR Spectroscopy* **1983**, *15*, 1-104.
22. Ikeda, T.; Matsumura, F.; Benjamin, D.M. *Science* **1977**, *197*, 497-499. Ikeda, T.; Matsumura, F.; Benjamin, D.M. *J. Chem. Ecol.* **1977**, *3*, 677-694.
23. Abad, A.; Arnó, M.; Doningo, L.R.; Zaragoza, R.J. *Tetrahedron* **1985**, *41*, 4937-4940.
24. Wenkert, E; Buckwalter, B.L. *J. Am. Chem. Soc.* **1972**, *94*, 4367-4369. Cambie, R.C. *J. Org. Chem.* **1975**, *40*, 3789-3791.
25. Matsubara, Y.; Zhou, Z.; Takekuma, S.; Nakano, T.; Huang, X; Morita, M. *Yukagaku* **1990**, *9*, 499-501.
26. McMurray, J.E.; Isser, S.J. *J. Am. Chem. Soc.* **1972**, *94*, 7132-7137.
27. Joseph-Nathan, P.; Santillan, R.L.; Schmitt, P.; Günther, H. *Org. Magn. Reson.* **1984**, *22*, 450-453. Oppolzer, W.; Godel, T. *Helv. Chim. Acta* **1984**, *167*, 1154-1167.
28. Ilofft, P.M.; Mirov, N.T. *J. Am. Pharm. Assoc.* **1954**, *43*, 373-378. Mullin, L.J.; Janet, M.; John, J.W. *Phytochem.* **1988**, *27*, 1731-1734.
29. Khan, V.A.; Salenko, V.L. *Khim. Prir. Soedin.* **1990**, 629-632.
30. Shirahama, H.; Osawa, E.; Chabra, B.R.; Shimokawa, T.; Yokono, T.; Kanaiwa, T.; Amiya, T.; Matsumoto, T. *Tetrahedron Lett.* **1981**, *22*, 1527-1528. Goweiss, A.; Kashman, Y. *Tetrahedron* **1983**, *39*, 3385-3396. Schneider, H.-J.; Agrawal, P.K. *Magn. Reson. Chem.* **1986**, *24*, 718-722. Krebs, H.C.; Rakotoarimanga, J.V.; Habermehl, G.G. *Magn. Reson. Chem.* **1990**, *28*, 124-128.
31. Perry, J.P.; Sanchez, X.M. *Nav. Stores Rev.* **1986**, *96*, 18-19. Ueda, N.; Dewa, N.; Tomoyuki, T.; Kageyama, M.; Nakashima, A.; Yamamoto, J.; Nakashima, R. *Chem. Express* **1989**, *4*, 813-816.
32. Coll, J.C.; Bowden, B.F. *J. Nat. Prod.* **1986**, *49*, 334-336. Pelletier, S.W.; Chokski, H.P.; Desai, H.K. *J. Nat. Prod.* **1986**, *49*, 892-900.
33. In spite of repeated extractions and washings, traces of acids **1** and **6** (2 mg each) were isolated from the most polar fractions of the "neutral material" (0.53 g).