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TERPENOIDS OF LODGEPOLE PINE BARK

J W. ROWE, R C RONALD* and B. A NAGASAMPAGI†

Forest Products Laboratory‡, Forest Service, U S Department of Agriculture, Madison, Wisconsin 53705, U S A

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Abstract—Additional extractives of lodgepole pine bark include pimaradiene, γ-cadinene, oplopanone (I), and the new natural products, 21-episerratenediol 21-methyl ether (IIa) and 18-norpimara-8(14),15-dien-4-ol (III) The structure of IIa was proved by converting it to 21β-methoxy-14-serraten-3-one (IIc) and to 21-episerratenediol dimethyl ether (IIb) The structure of III was proved by converting it and dihydropimaric acid (IVa) to 18-norpimar-8(14)-en-4-ol (IVb) The corresponding 19-norpimar-8(14)-en-4-ol (IVc) was also prepared

INTRODUCTION

THE BENZENE extract of lodgepole pine (*Pinus contorta* Dougl.) bark has been reported[1,2] to contain waxes, fats, sterols,[3] 13-epimanool, 13-epitorulosol, agathadiol, and isoagatholal.[4] We conclude our study of this species by reporting the third isolation of the unusual rearranged cadalenic sesquiterpene, oplopanone (I), the first isolation of a new triterpene, 21-episerratenediol 21-methyl ether (IIa);[5] and the first isolation of a new diterpene, 18-norpimara-8(14),15-dien-4-ol (III).[6,7] Pimaradiene and γ-cadinene are also present

RESULTS AND DISCUSSION

A sesquiterpene isolated from the benzene extract was shown to be identical to oplopanone by undepressed m m p , superimposable NMR and IR spectra, and correspondence

* Undergraduate student trainee at the U S Forest Products Laboratory, summer of 1965 Now National Academy of Sciences Overseas Research Fellow at the Universidade de Sao Paulo, Brazil

† Wisconsin Section of the American Chemical Society Postdoctoral Fellow Present address National Chemical Laboratory, Poona 8, India

‡ Maintained in cooperation with the University of Wisconsin

[1] J W ROWE and J H SCROGGINS, *J Org Chem* **29**, 1554 (1964), J W ROWE and G W SHAFFER, *Tetrahedron Letters* 2633 (1965), J W ROWE and G W SHAFFER, *Tetrahedron Letters* 2528 (1967)

[2] Others investigating the benzene extract of lodgepole pine bark (private communications) have obtained yields of only about 4% containing only small amounts of 13-epimanool Thus it would appear that our sample was unusual, perhaps infected by a fungus such as *Atropellis piniphila* that in lodgepole pine wood causes an increase in the alcohol benzene (1 2) extractives from 2 3 to 23 6% [K HUNT and A KUECHLER, *Bi-Mon Res Notes Canadian Forestry Serv* **26**(6), 59 (1970)]

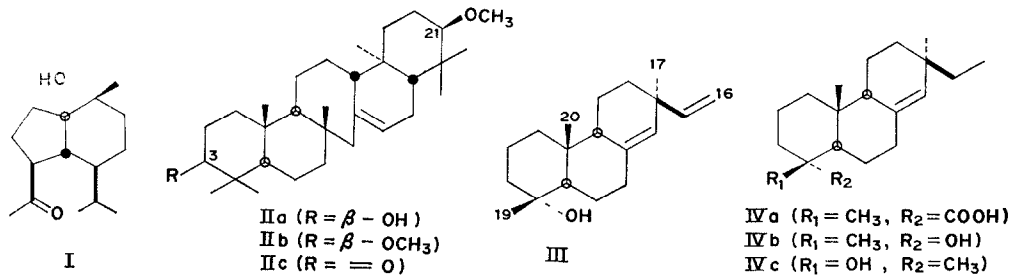
[3] J W ROWE, *Phytochem* **4**, 1 (1965)

[4] This compound was originally called agatholal (contortolal) [1] However, as pointed out by L MANGONI and R CAPUTO, *Tetrahedron Letters* 673 (1967) in their reference 7, this name had been used for the compound with the functional groups reversed

[5] We have also found this in western white pine (*Pinus monticola*) bark as one of the minor triterpenes [0 05% of the benzene extract, m p 250 5–251 5°, $[\alpha]_D^{22}$ −44 5° (*c* 0 8)] It is also identical with the methoxy-triterpenol reported in jack pine (*P banksiana*) bark[3] (0 01% of the benzene extract)

[6] Nomenclature is based on *The Common and Systematic Nomenclature of Cyclic Diterpenes*, 3rd rev , Oct 1968, with addenda and corrigenda of Feb 1969, available from the Forest Products Laboratory, Madison, Wisconsin 53705, U S A

[7] This compound also has been found as a minor component (0 003% of the benzene extract) of jack pine (*Pinus banksiana*) bark J W ROWE and C L BOWER, unpublished results



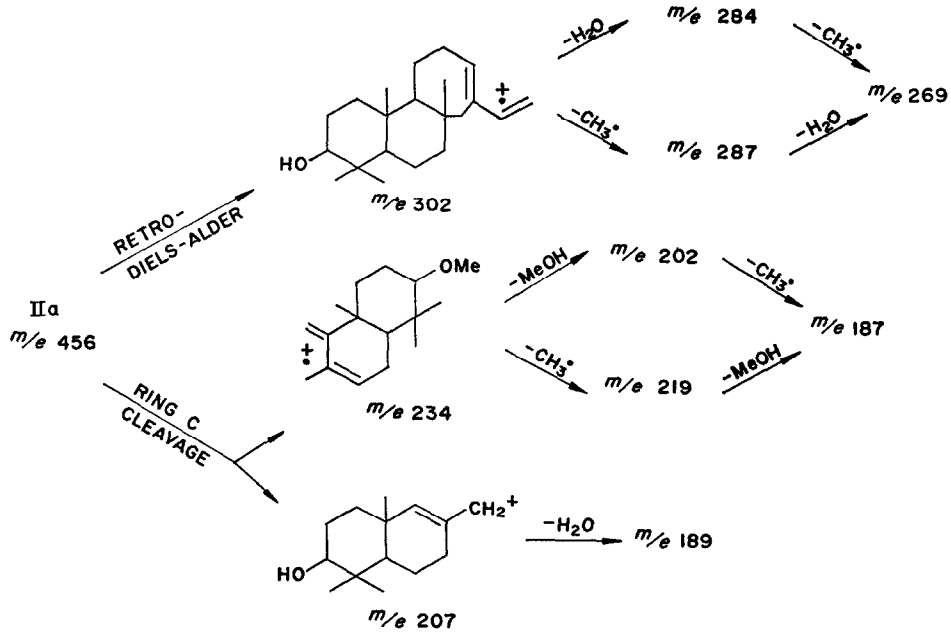
in other physical and spectral properties Oplopanone was first found in the shrub, *Oplopanax japonicus*[8] and later reported in the oleoresin of *Picea ajanensis* [9] The conversion of oplopanone into an antipyretic and antitussive drug has been described [10]

A high-melting crystalline material was isolated next The spectral data showed seven tertiary methyls, an equatorial secondary hydroxyl, an axial secondary methoxyl, and a trisubstituted double bond with an adjacent methylene This suggested the triterpenes, 21-episerratenediol 21-methyl ether (IIa) or 3-episerratenediol 3-methyl ether, neither of which was a known natural product The mass spectrum was typical of 14-serratenes,[11] and in particular was very similar to that of 21-episerratenediol and its dimethyl ether The diagnostic fragments are shown below and indicate that this triterpene was indeed 21-episerratenediol 21-methyl ether As expected, fragmentation to yield *m/e* 207 and its *m/e* 189 satellite predominates

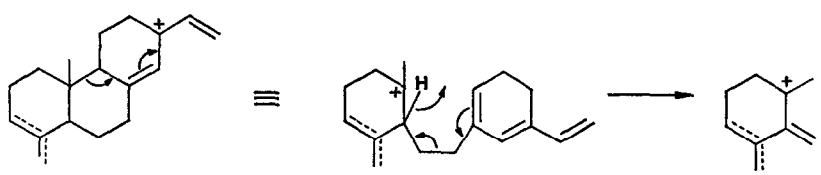
The structure of IIa was confirmed by methylation to yield 21-episerratenediol dimethyl ether (IIb) identical to an authentic sample [12] To verify the position of the hydroxyl, the triterpene was oxidized to 21β-methoxy-14-serraten-3-one (IIc) the positive ORD curve of which proves that the hydroxyl is at the 3 rather than the 21 position [13] Both IIb and IIc have recently been found as natural products [14,15]

Next to be isolated were white crystals, the GLC and TLC of which suggested a diterpene alcohol The IR spectrum showed characteristic vinyl and tertiary hydroxyl bands, and the NMR was identical to that of pimarol except that the 18-CH_2OH was missing The lack of an upfield shift of the 20-methyl through deshielding by hydroxyl[16] indicated that the hydroxyl should be 4α (equatorial) in analogy with the known natural products, 18-norabieta-8,11,13-trien-4-ol,[17] 18-norisopimara-7,15-dien-4-ol,[18] and 18-norisopimara-8(14),15-

[8] K TAKEDA, H MINATO and M ISHIKAWA, *Tetrahedron* Suppl No **11**, 219 (1966)
[9] V A BABKIN, ZH V DUBOVENKO and V A PENTEGOVA, *Izv Sib Otd Akad Nauk SSSR, Ser Khim Nauk* **1**, 168 (1970)
[10] H MINATO, *Jap Pat* 68 14, 211, *Chem Abs* **70**, 37943f (1969)
[11] J P KUTNEY, G EIGENDORF and I H ROGERS, *Tetrahedron* **25**, 3753 (1969)
[12] J P KUTNEY, I H ROGERS and J W ROWE, *Tetrahedron* **25**, 3731 (1969)
[13] Y TSUDA, T SANO, K KAWAGUCHI and Y INUBUSHI, *Tetrahedron Letters* 1279 (1964)
[14] 21-Episerratenediol dimethyl ether has been isolated for the first time as a natural product in 0 02% yield from the benzene extract of western white pine (*Pinus monticola*) bark Unpublished results, J W ROWE, M A LINTNER, B A NAGASAMPAGI and A H CONNER
[15] 21β-Methoxy-14-serraten-3-one was compared with a sample, m p 271–272°, isolated by I H ROGERS from Sitka spruce (*Picea sitchensis*) bark (unpublished), they were identical by TLC, GLC, IR, NMR, MS, and undepressed m m p It is also identical by ORD, NMR, IR, TLC, GLC and undepressed m m p to a compound isolated in 0 01% yield from the benzene extract of jack pine (*Pinus banksiana*) bark
[16] G HUGEL, A C OEHLSCHLAGER and G OURISSON, *Tetrahedron* Suppl No 8, 203 (1966)
[17] J W ROWE, B A NAGASAMPAGI, A W BURGSTAHLER and J W FITZSIMMONS, *Phytochem* **10**, 1647 (1971)
[18] P K GRANT, C HUNTRAKUL and D R J SHEPPARD, *Austral J Chem* **20**, 969 (1967)



dien-4-ol [19] This suggested the structure of 18-norpimara-8(14),15-dien-4-ol (III). The mass spectrum supported this postulated structure. The parent peak at *m/e* 274 was accompanied by a peak corresponding to the loss of water at *m/e* 256 and another corresponding to the subsequent loss of the doubly allylic 17-methyl to give the very strong *m/e* 241 peak This then fragments as is shown to give the very strong *m/e* 121 peak characteristic of 18(19)-oxygenated pimar-8(14)-ene derivatives.[20] The appearance of a parent peak and of a dehydration peak of only moderate intensity is characteristic of cyclic equatorial tertiary alcohols [21]



The structure was proved by hydrogenation to yield 18-norpimara-8(14)-en-4-ol (IVb) that was identical with the major alcohol produced when dihydropimaric acid (IVa) was oxidatively decarboxylated with lead tetraacetate The epimeric 19-norpimara-8(14)-en-4-ol (IVc) was also isolated as a minor product of this reaction Another norpimarane, 19-norpimara-8(14), 15-dien-3-one, has been isolated from *Pinus sylvestris* bark.[22]

[19] H H Quon and E P Swan, *Can J Chem* **47**, 4389 (1969)
[20] C R Enzell and R Ryhage, *Arkiv Kemi* **23**, 367 (1965)
[21] N S Wulfson, V I Zaretskii and I V Torgov, *4th Int Sympos Chem of Natural Products*, Stockholm, June (1966)
[22] T Norin and B Winell, *Acta Chem Scand* in press

To complete our examination of the extractives of this bark, the hydrocarbon fraction of the benzene extract was briefly investigated by Dr Lars Westfelt, Royal Institute of Technology, Stockholm, Sweden, and Dr Ernst bon Rudloff, Prairie Regional Laboratory, Saskatoon, Canada The major component appeared to be pimaradiene Several minor sesquiterpenes are present of which γ-cadinene ($[\alpha]_D^{22}$ +118°, n_D^{22} 1 5082, NMR and IR identical to an authentic sample) predominates

EXPERIMENTAL

M ps are measured in evacuated capillaries and are corrected Rotations are measured in $CHCl_3$ and NMR spectra in $CDCl_3$ Hydroxyl bands in the IR are accurately measured on a calibrated Beckman DK-2 spectrophotometer UV spectra are taken on a purged Cary 14 spectrophotometer Letters in parentheses are the designation of the IR and NMR spectra deposited with the Scientific Documentation Center

Isolation of oplopanone (I) A portion (1 78 g) of the 'contortolal'-containing fraction[1,4] obtained on chromatography of the unsaponifiables of the benzene extract was rechromatographed on silica gel in a multibore column Benzene–ether 4 1 eluted 132 mg of white crystals, which were recrystallized from hexane to constant m p 96–97°, $[\alpha]_D^{21}$ −16° (*c* 0 9) Lit m p 96–97°, $[\alpha]_D^{25.5}$ −20° (*c* 0 6) [8] A mixture m p was undepressed GLC (DEGS, SE-30) showed only a single peak, and TLC (silica) showed only a single spot No color was produced with tetranitromethane

The UV spectrum showed λ_{max}^{MeOH} 279 nm (ϵ = 38) and a very low end absorption at 210 nm The ORD ($CHCl_3$) had a negative Cotton effect centered at 285 nm with a molecular amplitude of −56, Lit 282 nm, −62 [8] The IR had a band at 3609 (CCl_4) for a tertiary hydroxyl and a carbonyl band at 1700 (KBr) or 1709 (CS_2) cm^{-1} The NMR and IR spectra were superimposable on those of oplopanone The mass spectrum showed the parent peak at *m/e* 238 (4%) that loses water to give *m/e* 220 (6%) To a small extent this loses CH_3 to give *m/e* 205 (3%) The base peak is acetyl, *m/e* 43 (100%) The major fragmentation ions are *m/e* 177 (21%), 153 (64%), 135 (42%), 71 (20%), 111 (14%), and the allyl ion at *m/e* 41 (20%) The only other significant ions are a series of weak peaks between *m/e* 55 and 109, none of which is more than 12% of the base peak

Isolation of 21-*episerratenediol* 21-*methyl ether* (IIa) *and* 18-*norpimara*-8(14),15-*dien*-4-*ol* (III) The 'iso-epimanool' fraction[1] (11 g) was chromatographed on 1 kg of alumina-40% $AgNO_3$ The chromatogram was developed by gradient elution with petrol (petroleum ether) and Et_2O

The first fractions yielded 580 mg of a yellow oil from which 121 mg of white crystals of IIa were obtained on crystallization from methanol Subsequent recrystallization to constant m p from CH_2Cl_2–MeOH and CH_2Cl_2–hexane yielded needles of 21-*episerratenediol* 21-*methyl ether*[5] pure by TLC (SiO_2, Al_2O_3) and GLC (SE-30), m p 250 5–252°, $[\alpha]_D^{21}$ −43 5° (*c* 0 9) (Found C, 81 63, H, 11 49, MeO, 6 83 $C_{31}H_{52}O_2$ required C, 81 52, H, 11 48, MeO, 6 79%) The NMR (BWLA) had a broad multiplet at τ 4 68 (1H, C=CH), a broad multiplet at 3 2 (1H, HOCH axial), a sharp singlet at 6 68 (3H, axial MeO), a triplet at 7 16 (1H, *J* = ~2 Hz, MeOCH equatorial), and seven sharp singlets for tertiary methyls at 9 04, 9 10, 9 12, 9 17, 9 20, 9 23 and 9 31 The IR (BWJE) had $\nu_{max}^{CCl_4}$ 3630 (equatorial OH) and ν_{max}^{KBr} 1094 (C—O—C) and 1626 and 792 cm^{-1} (C=CH) The UV had λ_{max}^{EtOH} 201 5 nm (ϵ 6700)

Pure ether then eluted 4 8 g of a complex mixture containing wax alcohols MeOH (1%) in ether then eluted 738 mg of crystals that were crystallized three times from hexane to yield needles of 18-*norpimara*-8(14),15-*dien*-4-*ol*[7] of constant m p 119–121°, $[\alpha]_D^{22}$ +92° (*c* 1 1) (Found C, 82 73, H, 11 71 $C_{19}H_{32}O$ required C, 82 54, H, 11 66%) The NMR showed three tertiary methyls at τ 8 84 (C-19), 9 00 (C-17), and 9 29 (C-20), pimaradiene-type unsaturation, 4 83 (1H, s, $W_{1/2}$ = 4 Hz), and 4 32 and 5 05 (3H, AB_2, *J* = 14 5 Hz) The IR has $\nu_{max}^{CCl_4}$ 3614 (tertiary OH) and ν_{max}^{KBr} 3077, 1634, 1401, 964 and 924 cm^{-1} (vinyl)

21-*Episerratenediol dimethyl ether* (IIb) KO*t*-Bu (8 g) was added to 50 mg of 21-episerratenediol 21-methyl ether (IIa) in 100 ml of anhydrous ether, the flask flushed with N_2, and the mixture kept overnight at room temp MeI (10 ml) was then added, the flask flushed with N_2, and the mixture kept at room temp. for 2 days and was occasionally shaken The mixture was then poured into H_2O and extracted as usual with ether to yield 59 mg of product that was chromatographed on 3 g of silica gel

Benzene eluted 40 mg of 21-episerratenediol dimethyl ether, pure by GLC (SE-30) and TLC (SiO_2), that was crystallized from benzene to yield colorless crystals, m p and m m p 298–300°, $[\alpha]_D^{20}$ −23° (*c* 1) [Lit 272–278° (Kofler block u c), $[\alpha]_D^{20}$ −16 4°,[12] 298–300°, $[\alpha]_D^{18}$ −22°] [14] The compound was identical with an authentic sample by TLC, GLC, IR and NMR Further elution with benzene yielded 18 mg of recovered IIa

21β-*Methoxy*-14-*serraten*-3-*one* (IIc) 21-Episerratenediol (IIa) (53 mg) was oxidized with Jones reagent in acetone[23] to yield 62 mg that were chromatographed on 3 g of alumina Petrol–benzene (10 1) eluted 44 mg

[23] R G CURTIS, I HEILBRON, E R H JONES and G F WOODS, *J Chem Soc* 457 (1953)

of white crystals of 21β-*methoxy*-14-*serraten*-3-*one* [15] This was crystallized from CH_2Cl_2–MeOH and CH_2Cl_2–hexane to a constant m p 283–283 5°, $[\alpha]_D^{22}$ −6° (*c* 0 8) (Found C, 81 91, H, 11 20, MeO, 6 88 $C_{31}H_{50}O_2$ required C, 81·88, H, 11 08, MeO, 6 83%) The IR (BWJG) had ν_{max}^{KBr} 1709 (C=O), 1095 (MeO), and 1630 and 796 (C=CH) cm^{-1} The UV had λ_{max}^{isooct} 294 nm (ϵ = 54) and a high end absorption, ϵ_{200} = 6500 The NMR (BWKY) had a broad multiplet at τ 4 65 (1H, C=CH), a sharp singlet at 6 69 (3H, axial methoxyl), a triplet at 7 16 (1H, *J* = 2 5 Hz, MeOCH equatorial), a multiplet at 7 6 (2 H, CH_2CO), and seven tertiary methyls at 8 93, 8 97, 9 09, 9 13, 9 13, 9 13 and 9 32 The ORD curve had a positive Cotton effect centered at 293 nm with a molecular amplitude of +51 The ORD in $CHCl_3$ (*c* 0 145) measured $[\alpha]_{589}$ −10°, $[\alpha]_{394}$ 0°, $[\alpha]_{312}$ +466° (max), $[\alpha]_{294}$ 0° and $[\alpha]_{270}$ −646° (min) The mass spectrum showed the parent ion at *m/e* 454 (base peak) with satellites at *m/e* 439 (M–Me), 422 (M–MeOH), and 407 (M–Me–MeOH) The next strongest high-mass peak was at *m/e* 300 corresponding to retro-Diels–Alder cleavage Cleavage through ring C yields the A/B ring fragment at *m/e* 205

Hydrogenation of 18-*norpimara*-8(14),15-*dien*-4-*ol* (III) III (40 mg) in 10 ml EtOAc was added to 6 mg of pre-reduced PtO_2 in 10 ml of EtOH and hydrogenated until rapid absorption of H_2 ceased The product was crystallized from hexane to constant m p to yield 18-*norpimar*-8(14)-*en*-4-*ol* (IVb), m p 80–81, 93–94° (dimorphous), $[\alpha]_D^{21}$ +15° (*c* 0 8) (Found C, 82 88, H, 11 34 $C_{19}H_{32}O$ required C, 82 54, H, 11 66%) The NMR showed τ 4 75 (1H, s, $W_{1/2}$ = 4 Hz, C=CH) 8 86 (3H, s, 19-Me), 9 15 (3H, s, 17-Me), 9 27 (3H, s, 20-Me) and 9 13 (3H, d, *J* = 6 Hz, 16-Me)

Oxidation of dihydropimaric acid (IVa) To a solution of 1 g of dihydropimaric acid in 30 ml of benzene plus 2 5 ml of pyridine under N_2 were added 1 95 g of $Pb(OAc)_4$ The reaction mixture was refluxed for 3 hr, cooled, filtered through Celite, and extracted as usual The resulting mixture of acetates and hydrocarbons was reduced with 2 7 g of $LiAlH_4$ in 150 ml of ether After refluxing for 2 hr, the excess $LiAlH_4$ was destroyed with EtOAc, and 50 ml of a saturated solution of Rochelle salt was added The mixture was extracted as usual to give 832 mg of a mixture that was chromatographed on 25 g of alumina

Petrol eluted 551 mg of a mixture of 19-norpimaradienes Petrol–benzene (10 1) eluted 10 mg of a complex mixture followed by 10 mg of pure (TLC, SiO_2) 19-*norpimar*-8(14)-*en*-4-*ol* (IVc) This was followed by 21 mg of a mixture of IVc and IVb Petrol–benzene (4 1) then eluted 133 mg of crude 18-norpimar-8(14)-en-4-ol (IVb)

IVc was crystallized once from hexane to yield crystals, m p 72–74°, $[\alpha]_D^{21}$ +30 5° (*c* 0 4) MW found by high resolution MS 276 244 $C_{19}H_{32}O$ required 276 245 The m s also showed very strong peaks corresponding to the loss of water (*m/e* 258), the axial allylic ethyl radical (*m/e* 247), and both (*m/e* 229) The last two were accompanied by satellites at *m/e* 248 and 230 corresponding to loss of the ethyl groups as ethylene with transfer of a 16-H to C-8 The only other strong high-mass peak was at *m/e* 121 corresponding to the characteristic ring A fragment (see Results and Discussion) The NMR showed τ 4 75 (1H, s, C=CH), 8 825 (3H, s, 18-Me), 9 07 (3H, s, 20-Me), 9 15 (3H, s, 17-Me) and 9 13 (3H, d, *J* = 6 Hz, 16-Me)

The center cut (110 mg) of crude IVb was chromatographed on alumina-40% $AgNO_3$ Subsequent to the elution of 15 mg of a mixture, petrol–benzene (17 3) eluted 70 mg of pure (GLC, SE-30, TLC, SiO_2, TLC, Al_2O_3–$AgNO_3$) 18-norpimar-8(14)-en-4-ol (IVb) that was crystallized 3 times from hexane, m p and mixed m p 93–94°, $[\alpha]_D^{18}$ +18° (*c* 0 9) It was identical with IVb derived from the natural product by, IR NMR, TLC (SiO_2, Al_2O_3–$AgNO_3$) and GLC (SE-30)

Acknowledgements—The authors thank Dr D. F Zinkel, U S Forest Products Laboratory, for a sample of dihydropimaric acid, Dr Willhalm, Firmenich et Cie, Geneva, Switzerland, for the mass spectra of oplopanone and the norpimaradienol, Professor R B Bates, University of Arizona, Tucson, U S A , for noting that the NMR of our sesquiterpene was identical to that of oplopanone, Dr I H Rogers, Canadian Forest Products Laboratory, Vancouver, for a sample of 21-episerratenediol dimethyl ether, and Prof J P Kutney, University of British Columbia, Vancouver, for the mass spectra of the triterpenes and IVc

Key Word Index—*Pinus contorta*, Pinaceae, lodgepole pine, diterpenes, triterpenes.