THE NEUTRAL PART OF THE BARK OF *PINUS LUCHUENSIS* MAYER

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The neutral part of the acetone extract from the bark of *Pinus luchuensis* Mayer has been investigated and found to consist of alkanes (C_{22}-C_{34}) and triterpenes of serratene type. The triterpenes are 3β-methoxyserrat-14-en-21-one, serrat-14-en-3, 21-dione, 3β-hydroxyserrat-14-en-21-one, 3β-21α-dimethoxyserrat-14-ene and 3β-methoxyserrat-14-en-21α-ol.

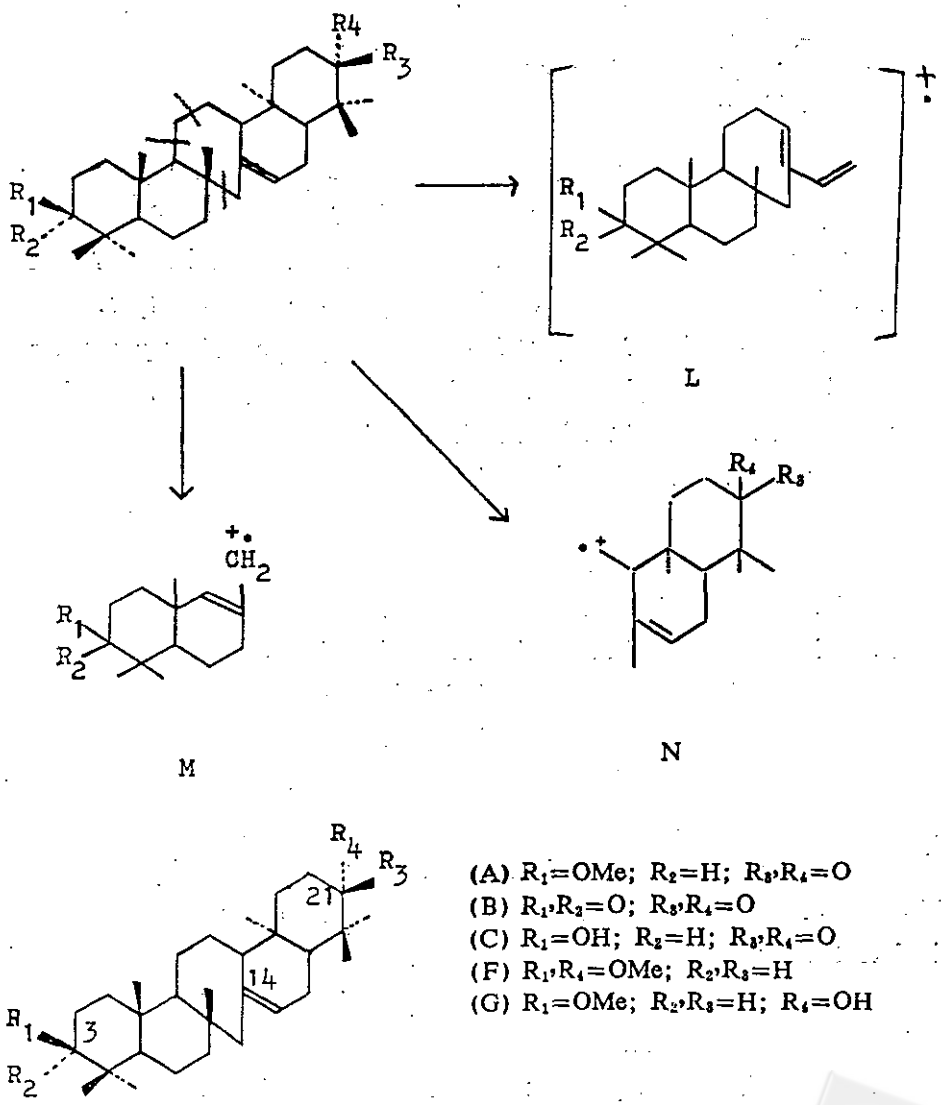
Triterpenes of the serratene type in which ring-C is seven membered, have been found in several species of Lycopodium[1], in a fern[2], in the bark of some Pinus[3] and Picea[4] species. We wish now to report the results of our investigations on the neutral part of the bark of *Pinus luchuensis* Mayer which contains the triterpenoids belong to the serratene type.

The Luchu pine (*Pinus luchuensis* Mayer) is native to Ryukyu and very popular in Taiwan. The chemical constituents of this plant have not been studied very well[5]. We investigated the neutral fraction of acetone extract of the bark of Luchu pine which was collected in the campus of National Taiwan University. The neutral fraction was triturated with *n*-hexane and *n*-hexane soluble portion was chromatographed through alumina column. The insoluble portion was chromatographed through silica gel column.

The first fraction from alumina column consists of small amount of *n*-alkanes (C_{22}-C_{34}) with an almost equal distribution of odd and even numbered chains.

Compound A was obtained from the second fraction of alumina column, *m.p.* 266°-267°. The compound also appeared from silica gel column of *n*-hexane insoluble part on elution with benzene. The empirical formula $C_{31}H_{50}O_2$ was established by elemental analysis and by mass spectrometry. The presence of an equtorial secondary methoxy group in the molecule was clear from the spectroscopic data: IR 1150 cm^{-1}, NMR 3-proton singlet at δ3.33 and one proton quartet at δ2.75 ($J_1=14$, $J_2=4$ H_z). The coupling constant in the signal patterns due to the proton geminal to the methoxy group indicates the presence of axial-axial and axial-equatorial couplings. So the methoxy group must posses the equatorial orientation. The presence of saturated carbonyl group was evident from the appearance of a strong IR absoption band 1706 cm^{-1}. Spectral evidence also indicated the presence of a trisubstituted double bond with an adjacent methylene (IR 800 cm^{-1}; NMR one proton multiplet δ5.43) and seven tertiary methyls (NMR 21 protons, δ1.10-0.73). The NMR spectrum is very characteristic for a serratene derivative which has only seven angular methyl groups whereas normal pentacyclic triterpenes posses eight such

groups. The mass spectrum has a very characteristic fragmentation pattern which has been summarized in a paper by Kutney *et al.*[6] The seven membered ring is the point of fragmentation and its cleavage gives rise to two main characteristic fragmentation (ion M and ion N), Fragments resulting from a retro Diels-Alder cleavage are found in a minor extent (ion L). The mass spectral fragmentation of compound A was that expected of a compound with a Δ^{14}-serratene skeleton. The cleavage fragment observed at *m/e* 221 (ion M, R_1=OMe, R_2=H, 90%) and 218 (ion N R_3, R_4=O, 88%) were suggested that this triterpene was 3β-methoxyserrat-14-en-21-one. The correctness of this assignment was proved by ORD measurement. The negative ORD curve of which proves that the ketone group is at the 21 rather than the 3-position[7]. The structure was shown to be correct by direct comparison with authentic sample. 3β-methoxyserrat-14-en-21-one was first isolated from the bark of pine by J. W. Rowe[7,8].



(A) R_1=OMe; R_2=H; R_3,R_4=O
(B) R_1,R_2=O; R_3,R_4=O
(C) R_1=OH; R_2=H; R_3,R_4=O
(F) R_1,R_4=OMe; R_2,R_3=H
(G) R_1=OMe; R_2,R_3=H; R_4=OH

Compound B which eluted from the alumina column after compound A, melted at 206°–208°, $C_{30}H_{50}O_2$. The spectral evidence indicated the presence of a carbonyl group, seven tertiary

methyls and a trisubstituted double bond. In the mass spectrum ions of type N (*m/e* 218, 76%) and M (*m/e* 205, 65%) were observed. The ketonic group gives rise to a negative Cotton effect. It was identified as serrat-14-en-3, 21-dione by direct comparison with the authentic sample, both gave superimposable IR spectra and undepressed mixed melting points. This compound has been obtained through oxidation of serratenediol[8], but no paper had reported its isolation from natural source.

Compound C which eluted from the alumina column after compound B melted at 267°-268°, $C_{30}H_{48}O_2$, the IR and NMR spectra showed the presence of seven angular methyls, a trisubstituted double bond, a carbonyl and a secondary hydroxyl group. The mass spectral fragmentation showed the satelite ions at 218 (ion N, R_3, R_4=O, 90%) and 207 (ion M, R_1=OH, R_2=H, 100%) of the Δ^{14}-serratene type[6]. The ketonic function gives rise to a negative Cotton effect typical of a C-21 keto group in a serratene system. Therefore this compound is a ketone with a C-21 keto group[7]. Acetylation yielded a monoacetate derivative. The NMR spectrum contained the olefinic proton multiplet, a sharp 3-proton singlet at δ2.08 arising from the acetate function and one proton multiplet centered at δ4.5 due to the secondary hydrogen geminal to the acetate group. The width of this signal (18 H_z) suggested that the acetate group is equatorial. The mass spectral fragmentation of acetate 218 (ion N, R_3, R_4=O, 70%) and 249 (ion M, R_1=OAc, R_2=H, 20%) also reassured the acetyl group at C-3 position. The identity of compound C acetate with 3β-acetoxyserrat-14-en-21-one was verified by direct comparison with the authentic sample. Both gave superimposable IR spectra and undepressed mixed melting points. We therefore assign the structure 3β-hydroxyserrat-14-en-21-one to compound C. It was first isolated from the barks of pine by J. W. Rowe[7,8].

Compound D and compound E were isolated from the last two fractions of alumina column. The spectra data of compound D indicated the presence of hydroxyl and carbonyl groups. The compound E is an alcohol. No further work had been performed due to the lack of pure material.

Compound G which obtained from the last fraction of silica gel column, melted at 318°-320°, $C_{31}H_{50}O_2$. The spectra properties indicated the presence of seven methyls, a trisubstituted double bond, a secondary methoxyl group and a hydroxyl group. The mass spectral fragmentation of this substance was that expected of a compound with a 14-serratene skeleton[6]. Thus besides the expected molecular ion peak at *m/e* 456, there were present important ionic fragments at 221 (ion M, R_1=OMe, R_2=H, 67%) and 220 (ion N, R_3=H, R_4=OH, 13%). Acetylation gave an acetate. The width of NMR signal at 4.57 (18 H_z) suggested that the acetate group is equatorial. Oxidation of compound G by chromium trioxide in pyridine yielded the corresponding ketone, *m.p.* 266°. It was identical in all respects with compound A and compound G is shown to be represented as 3β-methoxy-serrat-14-en-21α-ol[7]. The structure was shown to be correct by direct comparison with authentic sample.

Compound F was present in very low yield, isolated from the first fraction silica gel column, *m.p.* 292°-293°, $C_{32}H_{54}O_2$, and found to be structurally related to compound G and its spectral properties suggested a possible identity with compound G methyl ether. This compound was shown

to be identical with the methyl ether prepared from the compound G and therefore has the structure $3\beta, 21\alpha$-dimethoxyserrat-14-ene. The structure was shown to be correct by direct comparison with authentic sample.

EXPERIMENTAL

The IR spectra were recorded on a Perkin Elmer Model 700 instrument. NMR spectra were determined in $CDCl_3$ with TMS as internal standards on Varian Model T-60 instrument. The chemical shifts were shown in δ value (*ppm*). The mass spectra were measured on Hitachi RMS-4 mass spectrometer. ORD curves were taken in $CHCl_3$ solution on a JASCO J-20 Automatic Recording Spectropolarimeter. All *mps* were uncorrected. Plates for TLC were prepared using silica gel G as absorbent. The silica gel plates were impregnated with 7% $AgNO_3$ solution, dried and activated in the usual way. As developing solvents, chloroform and benzene: ethyl acetate (3:1) were used. In all cases, the spray reagent of choice was antimony pentachloride dissolved in CCl_4 (20%). The spots became visible after spraying or heating the sprayed plate at 120°.

The air-dried bark (1.2 *kg*) was extracted with acetone for several times. The extract was taken to dryness to provide a crude mixture in a yield of 1.9%. The mixture was dissolved in ether and the ether soluble part was treated with aq. Na_2CO_3 to remove the acidic constituents (4.6 *g*). The remaining neutral fraction (16.1 *g*) was triturated with *n*-hexane and separated into a *n*-hexane soluble fraction (A, 11.2 *g*) and *n*-hexane insoluble fraction (B, 4.4 *g*). The *n*-hexane soluble part was separated on a neutral alumina column with *n*-hexane, *n*-hexane-benzene, benzene, benzene-ethyl acetate as eluent. The separation was followed by TLC and the fractions were combined in a suitable manner to give fraction A-I～A-VII. The *n*-hexane insoluble part (4.4 *g*) was dissolved in the minimum quantity of ether. To the solution a few grams silica gel was added and the ether was evaporated off from the slurry. The silica gel was then transferred to the top of a column filled with silica gel in *n*-hexane. Elution with *n*-hexane, benzene followed by benzene-ethyl acetate provided the various fraction (B-I～B-IV) in a fair state of purity.

The A-I fraction (0.8 *g*) consisted of hydrocarbons. They were separated into saturated and unsaturated fractions by column chromatography on silver nitrate impregnated silica gel with *n*-hexane as eluent. The saturated fraction (0.31 *g*) consisted of alkanes which were analyzed by GLC (5% carbowax 20 *M* and 5% SE-30) and coinjection with authentic samples. It was shown to be a complex mixture of most *n*-alkanes with the following composition:

Compound	$C_{22}H_{46}$	$C_{23}H_{48}$	$C_{24}H_{50}$	$C_{25}H_{52}$	$C_{26}H_{54}$	$C_{27}H_{56}$
%	3.5	3.5	5.6	7.3	8.3	10.3
Compound	$C_{28}H_{58}$	$C_{29}H_{60}$	$C_{30}H_{62}$	$C_{31}H_{64}$	$C_{32}H_{66}$	$C_{33}H_{68}$	$C_{34}H_{70}$
%	13.5	12.5	12.1	10.7	9.0	2.8	1.1

The unsaturated fraction (0.45 *g*) was found to be a complex mixture and was not further investigated.

Isolation of compound A:

Compound A was isolated from fraction A-II (2.2 *g*) and B-III (1.6*g*) after rechromatography on alumina and then recrystallization from ethanol. TLC showed only a single spot. *mp.* 266°-267°, found C, 82.04; H, 11.06; $C_{31}H_{50}O_2$ required: C, 81.88; H, 11.08. The IR had ν_{max}^{KBr} 1706 (>C=O), 1105 (-OCH_3) and 800 cm^{-1}. The NMR had a multiplet at 5.43 (1H, C=CH), a singlet at 3.33 (3H, -OCH_3), a quartet at 2.75 (1H, $J_1=14$; $J_2=4$ H_z, MeOCH axial) and seven methyls at 1.10-0.73. The ORD (C, 0.013) curve had a negative Cotton effect $[\phi]_{350}=-681$, $[\phi]_{312}=-2043$, $[\phi]_{275}=+1502$, molecular amplitude $=-35.4$; Mass spectrum: fragments at *m/e* 454, 439, 422, 407, 221 and 218. Compound A was identified as 3β-methoxyserrat-14-en-21-one by comparison with an authentic sample (IR and mixed *m.p.*).

Isolation of compound B:

Compound B was isolated from fraction A-III (1.4 *g*). It was crystallized from benzene. *mp.* 206°-208°, found: C, 82.43; H, 10.77; $C_{30}H_{46}O_2$ required: C, 82.13; H, 10.57. The IR had ν_{max}^{KBr} 1710 cm^{-1} (>C=O) NMR had a multiplet at 5.43 and seven methyls at 1.0-0.91. The ORD (C, 1) curve had a negative Cotton effect, $[\phi]_{400}=-43$, $[\phi]_{325}=-354$, $[\phi]_{300}=+631$, molecular amplitude $=-9.8$. Mass spectrum: fragments at 438, 423, 218 and 205. Compound B was identified as serrat-14-en-3, 21-dione by comparison with an authentic sample (IR and mixed *m.p.*).

Isolation of compound C:

Compound C crystallized from A-IV (2.1 *mg*). Recrystallization from benzene yielded white needles *m.p.* 267°-268°. Found C, 82·01; H, 10.81 $C_{30}H_{48}O_2$ required: C, 81.76; H, 10.98. The IR had ν_{max}^{KBr} 3490 (-OH), 1705 (>C=O) and 795 cm^{-1} (>C=CH). The NMR had a multiplet at 5.42 (1H, C=CH), a multiplet at 3.16 (1H, H-C-OH) and seven tertiary methyls at 1.10, 1.03, 0 97, 0.90, 0.82, 0.80 and 0.77. The ORD (C, 0.4) curve had a negative Cotton effect, $[\phi]_{350}=-831$, $[\phi]_{314}=-2122$, $[\phi]_{275}=+477$, molecular amplitude $=-26.0$. Mass spectrum fragments observed at *m/e* 440, 425, 422, 407, 218, and 207.

Acetylation of compound C:

A sample of compound C (27 *mg*) was dissolved in dry pyridine (5 *ml*) and reacted with acetic anhydride (0.75 *ml*) at room temperature for seven days. Poured the reaction mixture into water (30 *ml*) and boiled to decompose the excess of acetic anhydride. Allowed to stand for a while, filtrated the residual insoluble acetyl derivative and washed it with a small amount of cold dilute HCl then water. Recrystallization from acetone got white plates (24 *mg*) *m.p.* 325°-327°. IR ν_{max}^{KBr} 1725 and 1240 (-OAc) and 1705 (>C=O), NMR 5.42 (1H, multiplet, C=H) 4.50 (1H, multiplet, H-O-Ac, $\frac{1}{2}$ height width 18 H_z) 2.08 (3H, singlet, -OAc), 1.10-0.83 (21H -CH_3). Mass spectrum *m/e* 482, 467, 422, 407, 249 and 218. It was identified as 3β-acetoxy-serrat-14-en-21-one by comparison with an authentic sample (IR and mixed *m.p.*).

Isolation of compound D:

Compound D was isolated from fraction A-VI (0.4 *g*). After rechromatography on silica

gel and then recrystallization from benzene *m.p.* 280°-283°. The IR spectrum was very similar to that of compound C, but TLC analysis showed different R_f value. ν_{max}^{KBr} 3450 (-OH), 1705 ($\rangle$C=O) and 795 cm^{-1} (C=CH).

Isolation of compound E:

Compound E was obtained from fraction A-VII (1.1 *g*). The attemps to purify the fraction A-VII by further chromatography were difficult. It was complex mixture of similar compounds. After several chromatography and recrystallization, small amount of compound E was obtained. *m.p.* 224°-227°. ν_{max}^{KBr} 3350 (-OH) and 785 cm^{-1} (C=H).

Isolation of compound F:

Compound F crystallized from fraction B-I (0.04 *g*). It was recrystallized from benzene to yield white crystals, *m.p.* 292°-293°, pure by TLC, Found: C, 81.48; H, 11.55; $C_{32}H_{54}O_2$ required: C, 81.47; H, 11.49. IR ν_{max}^{KBr} 1105 (-CCH_3) and 790 cm^{-1} (C=CH). NMR: 5.40 (1H, multiplet, C=CH), 3.40 (6H, singlet, -OCH_3) 2.80, 2.60 (2H, quartets, J=4 H_z and 11 H_z H-C-OCH_3) 1.10-0.75 (21H, -CH_3). Mass spectrum: *m/e* 470, 455, 438, 423, 234 and 221.

Isolation of compound G:

Compound G was isolated from fraction B-IV (0.8 *g*). White crystals recrystallized from benzene, *m.p.* 318°-320°. found: C, 81.42; H, 11.48; $C_{31}H_{52}O_2$ required; C, 81.08; H, 11.37. IR ν_{max}^{KBr} 3400 (-OH), 1100 (-OCH_3) and 800 cm^{-1} (C=CH). NMR: 5.43 (1H, multiplet C=CH) 3.20 (1H, multiplet H-C-OH), 3.40 (3H, singlet -OCH_3) 2.80 (1H, multipplet H-C-OMe), 1.00-0.70 (21H, -CH_3). Mass spectrum: *m/e* 456, 441, 438, 221 and 220.

Acetylation of compound G:

A sample of the compound G (26 *mg*) was acetylated with acetic anhydride in pyridine at room temperature as described above to yield the acetate. IR ν_{max}^{KBr} 1730 and 1245 cm^{-1} (-OAc), NMR 5.40 (1H, multiplet C=CH), 4.57 (1H, multiplet, halfheight width 18 H_z, H-C-OAc), 3.40 (3H, singlet, -OCH_3), 2.02 (3H, singlet, -OAc) and 1.02-0.73 (21H, -CH_3). Mass spectrum: *m/e* 498, 438, 423, 262 and 221.

Methylation of compound G:

A 3-necked 50 *ml* flask was fitted with a magnetic stirrer, N_2 gas inlet tube, condenser with drying tube and a dropping funnel. Full precaution was taken to exclude moisture from the walls of the apparatus. Compound G 40 *mg* was dissolved in warm dry toluene (10 *ml*) and admitted to the reaction vessel via the dropping funnel. The appartus was then throughly purged with N_2 gas and freshly cut potassium metal (110 *mg*) was added. The flask was warmed (oil bath 80°) until the potassium was molten and stirring was commenced. After 2 *hrs* the reaction vessel was allowed to cool and an excess of pure dry methyl iodide (8 *ml*) was added from the dropping funnel. Stirring and heating were recommenced and the reaction was continued for a further 3 *hrs.* After cooling, the excess potassium was destroyed by the addition of an excess methanol. The solvent was evaporated and the reaction product was extracted with chloroform. The combined chloroform extractions were washed with water, dried with anhydrous sodium

sulfate and evaporated to yield the crude methyl ether. By chromatography through an alumina column with $CHCl_3$ as eluent, yielded crystals 26 *mg*. *m.p.* 292°–293°. The synthetic dimethyl ether was identical with the natural compound F (NMR, IR and TLC).

Oxidation of compound G:

Compound G (73 *mg*) was dissolved in dry pyridine (5 *ml*). This solution was added slowly to a slurry of chromium trioxide (141 *mg*) in dry pyridine (3 *ml*) and the reaction mixture was stirred at room temperature for two days, then allowed to stand for half an hour and filted under suction. The dark brown *ppt* was washed throughly with cold water and the crude ketone was then recovered by extraction with warm $CHCl_3$ (300 *ml*, in three portions). The solution was washed with dilute $HCl_{(aq)}$ and distilled water. By chromatography through alumina column with $CHCl_3$ as eluent, yielded 26 *mg*, *m.p.* 266°–267°. The IR, NMR and TLC data showed that the synthetic product was identical to compound A.

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REFERENCE

(1) Y. Inubushi, T. Sano and Y. Tauda, *Tetrahedron Letters*, 2959 (1964).
Y. Tsuda and T. Fryjimoto, *J. Chem. Soc. D*, 1042 (1969).
Y. Tsuda and M. Hatanaka, *J. Chem. Soc. D*, 1040 (1969).
R.H. Burnell, I. Mo and M. Moinas, *Phytochemistry.*, 2815 (1972).
(2) G. Berti, F. Bottari, A. Marsili, I. Morelli and A. Mandelbaum, *Chem. Comm.*, 50 (1967)
(3) J.W. Rowe, *Tetrahedron Letters.*, 2347 (1964).
(4) I.H. Rogers and L.R. Roson, *Can. J. Chem.*, 48, 1021 (1970).
J.P. Kutney and I.H. Rogers, *Tetrahedron Letters.*, 761 (1968).
J.P. Kutney, I.H. Rogers and U.W. Rowe, *Tetrahedron.*, 25, 3731 (1969).
(5) K.T. Wang, Dr. Sci. Thesis, Tohoku Univ. Sendai, Japan 85 (1962).
(6) J.P. Kutney and G. Eigendorf, *Tetrahedron*, 25, 3753 (1969).
(7) J.W. Rowe and C.L. Bower, *Tetrahedron Letters.*, 2745 (1965).
(8) J.W. Rowe, *Tetrahedron Letters.*, 2347 (1964).
Y. Inubashi, T. Sano and Y. Tsuda, *Tetrahedron Letters*, 1303 (1964).