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THREE NEW OXYGENATED TRITERPENOIDS OF THE LUPANE SERIES FROM *GYMNOSPORIA WALLICHIANA**

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Key Word Index—*Gymnosporia wallichiana*; Celastraceae; gymnosporic acid; wallichianic acid; wallichianol; β-amyrin; friedelin; 3β-hydroxy-29-norlupan-20-one; triterpenoids.

Abstract—Two new epimeric triterpenoid acids, gymnosporic acid, 3β-hydroxy-(20*R*)-lupan-29-oic acid, wallichianic acid, 3β-hydroxy-(20*S*)-lupan-29-oic acid and a new diol wallichianol, (20*S*)-lupane-3β,29-diol have been isolated from *Gymnosporia wallichiana*, in addition to β-amyrin, friedelin, 3β-hydroxy-29-norlupan-20-one and dulcitol.

INTRODUCTION

The alcoholic extractive of the plant *Gymnosporia wallichiana* exhibited an anticancer activity in the PS system [1]. So far no chemical work on this species is reported in the literature. A detailed chemical investigation on this plant was, therefore, undertaken.

RESULTS AND DISCUSSION

The alcoholic extractive was subjected to a solvent fractionation, whereby most of the anticancer activity resided in the $CHCl_3$ soluble fraction. TLC of this fraction showed the presence of ten constituents designated as A, B, C, D, E, F_1, F_2, G, H and I, out of which compounds B, C, D, F_1, G and H were isolated. Compounds B, C and D were identified as friedelin, β-amyrin and 3β-hydroxy-29-norlupan-20-one [2] respectively from their physicochemical data. Compounds F_1, G and H were new and will, hereafter, be referred to as wallichianol, gymnosporic acid and wallichianic acid respectively. The present communication deals with their structure elucidation.

Gymnosporic acid, $C_{30}H_{50}O_3$, (M^+ 458) mp 290°, $[\alpha]_D$ −44 showed IR bands at 3360–2500, 1695 cm^{-1} for a COOH group. On treatment with CH_2N_2 it furnished a monomethyl ester, $C_{31}H_{52}O_3$ (M^+ 472), mp 204°, $[\alpha]_D$ −40, ν_{max} 3440, 1035 (OH), 1720, 1185 (ester) cm^{-1}. Its PMR spectrum exhibited signals for six tertiary C-Me groups, a secondary C-Me, a C<u>H</u>-COOMe, a C<u>H</u>-O and a OMe group. Since the IR spectrum of the ester showed an OH bond, it was acetylated to give a monoacetate, $C_{33}H_{54}O_4$ (M-60 at *m/e* 454), mp 286°, $[\alpha]_D$ −48, ν_{max} 1730, 1245 (OCOMe), 1725, 1190 (COOMe) cm^{-1}, PMR: δ 2.04 (3H, *s*, OCOMe). The molecular formula and physiochemical data of gymnosporic acid and its derivatives thus indicated that it was a hydroxy triterpenoid acid.

The $LiAlH_4$ reduction of methyl gymnosporate furnished a diol (gymnosporol)†, $C_{30}H_{52}O_2$ (M^+ 444), mp 236°, ν_{max} 3400, 1035 cm^{-1} (OH), which exhibited a PMR signal for CH_2OH (2 × 1H, *d*, centred at 3.68 and 3.90 ppm) and formed a diacetate, mp 160°. The diol, on tosylation followed by $LiAlH_4$ reduction, yielded a product, $C_{30}H_{52}O$ (M^+ 428), mp 205°, which was identified as dihydrolupeol by comparison with the authentic sample. From the above sequence of reactions it was established that gymnosporic acid possessed a lupane skeleton bearing a 3β-OH.

The position of the COOH group was established as follows. In the MS of dihydrolupeol a prominent peak is observed at *m/e* 385, which is formed by the loss of the isopropyl side chain from the molecular ion. The same peak was also intense in the MS of gymnosporic acid, its Me ester and gymnosporol and must have resulted by the loss of the side chain from the molecular ion. The side chain must, therfore, carry the oxygen function. Further evidence was also forthcoming from a signal (*q*,*d*) for a Me-C<u>H</u>-COOR proton in the PMR spectra of methyl gymnosporate and its acetate. The structure of gymnosporic acid was, thus, established as 3β-hydroxy-lupan-29-oic acid and that of gymnosporol as lupane-3β,29-diol. The latter was also obtained by hydroboration [4] of lupeol, thus furnishing further proof of its structure.

Wallichianic acid, $C_{30}H_{50}O_3$ (M^+ 458), mp 280°, $[\alpha]_D$ 28°, also exhibited IR absorption bands at 3400–2605, 1700 cm^{-1} for a COOH group. On treatment with CH_2N_2 it formed a monomethyl ester, $C_{31}H_{52}O_3$ (M^+ 472), mp 212°, $[\alpha]_D$ 21°, which on acetylation furnished a monoacetate (IR: no OH band), $C_{33}O_{54}O_4$ (M^+ 514), mp 282°, $[\alpha]_D$ 31°. The IR, PMR and mass spectral data of wallichianic acid and its derivatives indicated that like gymnosporic acid, wallichianic acid, also contained a secondary OH, six tertiary C-Me groups and a Me-C<u>H</u>-COOR group. In a parallel sequence of reactions, methyl wallichianate, on $LiAlH_4$ reduction, gave a diol (wallichianol), $C_{30}H_{52}O_2$ (M^+ 444), mp 266°. The diol, on tosylation followed by $LiAlH_4$ reduction yielded dihydrolupeol. It was, therefore, confirmed that gymnosporic acid and wallichianic acid have the same gross structure. The only point of difference could be the configuration at C-20.

Křeček *et al.* [5] have established that in the C-29

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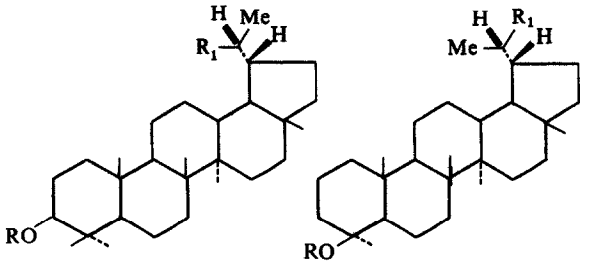
†Lupane-3β,29-diol, obtained by $LiAlH_4$ reduction of 3β-acetoxy lup-20 (29)-en-30-oate, has a mp 236–38° [3].

Table 1. Chemical shifts of the C-20 methyl group and J values between the C-20 and C-19β protons of gymnosporic acid (1) and wallichianic acid (2)

Compound	C-20 Me	$J_{20,19}$ (Hz)
1a	1.12	2.0
1b	1.10	2.0
2a	1.03	3.0
2b	1.03	3.0

oxygenated compounds of the lupane series, the 20R-epimer exhibits the C-20 methyl PMR signal at a higher field and the coupling constant between the C-20 proton and the C-19β proton has a lower value than those of the 20S-epimer. Table 1 shows that methylgymnosporate (**1a**) and its acetate (**1b**) have a higher value for the C-20 methyl signal and a lower value for the coupling constant between the C-20 proton and the C-19 proton than the corresponding values for methyl wallichianate and its acetate. Thus gymnosporic acid (**1**) was assigned the 20R-configuration and wallichianic acid (**2**) the 20S-configuration. These assignments were also in conformity with $\Delta\varepsilon$ value from the CD curves of methyl gymnosporate (-0.88 at 215 nm) and methyl wallichianate (1.83 at 215 nm)[4].



1. R = H; R_1 = COOH
1a. R = H; R_1 = COOMe
1b. R = Ac; R_1 = COOMe

2. R = H; R_1 = COOH
2a. R = H; R_1 = COOMe
2b. R = Ac; R_1 = COOMe

Compound F_1, $C_{30}H_{52}O_2$ (M^+ 444), mp 266° (diacetate, mp 218–20°), was identified as wallichianol by direct comparison of its physical data with those of the authentic sample obtained by $LiAlH_4$ reduction of methyl wallichianate.

The aqueous fraction of the alcoholic extractive on concentration yielded dulcitol, $C_6H_{14}O_6$, mp 189° identified by its comparison with the authentic sample.

EXPERIMENTAL

All mp's are uncorr. The PMR spectra were recorded at 60MHz in $CDCl_3$ unless otherwise stated. R_f values refer to TLC on Si gel plates. Solvent 1:C_6H_6–MeOH (49:1); Solvent 2: C_6H_{14}–Me_2CO (3:1). The alcoholic extractive of the plant (aerial parts, 16 kg, voucher specimen preserved in CDRI) was resolved into hexane, $CHCl_3$, n-BuOH and H_2O soluble fractions. The $CHCl_3$ soluble fraction showed ten spots viz. A,B,C, D,E,F_1,F_2,G,H,I on TLC. It was subjected to a gross frationation over Si gel by successive elution with hexane, C_6H_6, $CHCl_3$ and EtOAc. The C_6H_6 eluate on further chromatography over Si gel yielded compounds B, mp 266°, R_f 0.69; C, mp 200°, R_f 0.37 and D, mp 236°, R_f 0.25 (solvent 1). Compounds B and C were identified as friedelin and β-amyrin respectively by comparison of their physical data with those of the authentic samples and compound D as 3β-hydroxy-30-norlupan-2 one by comparison of its physical data with those reported in the literature [2]. The $CHCl_3$ eluate, on further chromatography over Si gel, using hexane–Me_2CO (8:1) as the eluant, first gave a mixture of compounds F_1 and F_2 and then pure compounds G and H (R_f 0.30 and 0.20, solvent 2) both of which crystallized from EtOH, mps. 290° and 280° respectively. The mixture of compounds F_1 and F_2 showed a single spot on Si gel TLC but two spots of very close R_f (0.51 and 0.46) on Si gel–$AgNO_3$ (9:1) TLC plates developed with C_6H_6–MeOH (49:1) and in its MS the M^+, M-15 and M-18 ions of compounds F_1 and F_2 differed by two mass units. Since compounds F_1 and F_2 could not be separated by any chromatographic method, the mixture (100 mg) was treated with Br_2 in $CHCl_3$ (100 ml) till the colour of Br_2 persisted. It was then evapd. The TLC of the product showed that compound F_1 was intact, whereas compound F_2 had changed to a mixture of products of higher R_f values, obviously by oxidation with Br_2. Compound F_1 was isolated from the reaction product by chromatography on Si gel in C_6H_{14}–Me_2CO (5:1) and crystallized from EtOH, mp 266°. The aq. fraction of the alcoholic extractive was concd and left overnight in the refrigerator whereby it deposited a colourless crystalline mass. It was filtered and recrystallised from EtOH, mp 189° and identified as dulcitol by comparison with the authentic sample.

Compound F_1 (wallichianol). Mp 266°, ν_{max} cm^{-1}: 3340, 2915, 2860, 1450, 1375, 1030. PMR (C_5D_5N): δ 0.70, 0.77, 0.85, 1.08 (3H each, *s*, 4 × Me), 0.90 (9H, *s*, 3 × Me), 3.30 (1H, *m*, C<u>H</u>$_2$OH), 3.55 (2H, *d*, J = 7Hz, C<u>H</u>$_2$OH). MS (*m/e*): 444 (M^+), 429 (M-15), 426 (M-18), 411 (M-18-15), 395, 385 (M-59), 376, 290, 275, 259, 247, 231, 221, 217, 207, 189, 177, 175, 163, 149, 135, 121. Wallichianol diacetate was prepared by Ac_2O and C_5H_5N, mp 218–20°; ν_{max} cm^{-1}: 1735, 1720, 1235. PMR ($CDCl_3$): δ 0.77, 0.92, 1.03, 1.08, (3H each, *s*, 4 = Me), 0.87 (9H, *s*, 3 × Me), 2.03 (6H, *s*, 2 × OCOMe), 3.83 (2H, *d*, J = 7Hz, C<u>H</u>$_2$OAc), 4.47 (1H, *m*, CHOAc).

Compound G (gymnosporic acid). Mp 290°, $[\alpha]_D$ −44 (*c* 1% $CHCl_3$–MeOH (3:1)), ν_{max} cm^{-1}: 3360–2593, 1695 (COOH), 2930, 2860, 1450, 1375 (*d*), 1020; MS: *m/e* 458 (M), 443 (M-15), 440 (M-18), 425 (M-18-15), 397, 385 (M-73), 367, 304, 289, 221, 207, 189, 163, 149, 135, 109, 107. (Found: C, 78.4; H, 10.73. $C_{30}H_{50}O_3$ requires: C, 78.54; H, 10.98%). Gymnosporic acid was methylated with CH_2N_2 to give methyl gymnosporate which crystallised from EtOH, mp 204° [α]$_D$ −44° (*c* 1% $CHCl_3$) ν_{max}^{KBr} cm^{-1}: 3440, 1035 (OH), 1720, 1185 (COOMe); PMR: δ 0.75, 0.77, 0.86, 0.90, 0.98, 1.05 (3H each, *s*, 6 × C-Me), 1.12 (3H, *d*, J = 7 Hz, C-Me), 2.78 (1H, *qd*, J = 7 and 2 Hz, Me-C<u>H</u>-COOMe, on 90 MHz machine), 3.2 (1H, *m*, C<u>H</u>OH), 3.65 (3H, *s*, OMe); MS: *m/e* 472 (M), 457 (M-15), 454 (M-18), 439 (M-15-18), 423, 411, 385 (M-87), 367, 352, 318, 303, 275, 249, 235, 207, 189, 177, 175, 163, 135, 121, 107. Methyl gymnosporate, on acetylation by Ac_2O–C_5H_5N, formed an acetate which crystallised from EtOH, mp 286°, $[\alpha]_D$ −48 (*c* 1%, $CHCl_3$), ν_{max}^{KBr} cm^{-1}: 1730, 1245 (OCOMe), 1725, 1190 (COOMe); PMR: δ 0.75, 0.86, 0.93, 1.05 (3H each, *s*, 4 × C-Me), 0.85 (6H, *s*, 2 × C-Me), 1.1 (3H, *d*, J = 7 Hz, C-Me), 2.04 (3H, *s*, OCOMe), 2.75 (1H, *qd*, J = 7 and 2 Hz, Me-C<u>H</u>-COOMe), 3.65 (3H, *s*, OMe), 4.48 (1H, *q*, J = 9 and 6 Hz, C<u>H</u>OAc); MS: *m/e* 454 (M-60), 439 (M-60-15), 427 (M-87), 411, 367 (M-87-60), 249, 235 and 189.

$LiAlH_4$ reduction of methyl gymnosporate. Methyl gymnosporate (75 mg) was refluxed with $LiAlH_4$ (50 mg) in THF for 3 hr. After usual work up, the product, gymnosporol, was purified by chromatography and crystallised from EtOH, mp 236°, ν_{max}^{KBr} cm^{-1}: 3400, 1035 (OH); PMR (C_5D_5N): δ 0.55, 0.67, 0.70, 0.78, 0.82, 0.98 (3H each, *s*, 6 × C-Me), 0.85 (3H, *d*, J = 7Hz, C-Me), 3.25 (1H, *m*, C<u>H</u>-OH), 3.68 and 3.90 (1H each *brd*, J = 15Hz, C<u>H</u>$_2$OH); MS: *m/e* 444 (M^+), 429 (M-15), 426 (M-18), 411 (M-15-18), 395, 385 (M-59), 367, 290, 275, 259, 247, 231, 222, 217, 207, 189, 177, 175, 163, 149, 135, 123, 121, 109, 107. Acetylation of gymnosporol by Ac_2O–C_5H_5N method yielded a diacetate mp 160°; ν_{max}^{KBr} cm^{-1}: 1745, 1735, 1250; PMR: δ 2.05, 2.07 (3H each, *s*, 2 × OCOMe), 3.87, 4.0 (1H each, *d*, J = 15 Hz, C<u>H</u>$_2$-OAc), 4.52 (1H, *m*, C<u>H</u>OAc).

Tosylation and $LiAlH_4$ reduction of gymnosporol. Gymnosporol (75 mg) was tosylated with PTSCl in C_5H_5N as usual. The crude

product (80 mg) was refluxed with $LiAlH_4$ (60 mg) in THF for 6 hr followed by usual work up. The product was purified by chromatography over Si gel and crystallised from EtOH, mp 205°. The product was identified as dihydrolupeol by direct comparison of its physical data with those of the authentic sample.

Hydroboration of lupeol. To a soln of lupeol (40 mg) in dry Et_2O (10 ml) was added $LiAlH_4$ (60 mg) and an ethereal soln of $BF_3.Et_2O$ (0.5 ml) at 0° under N_2 atmosphere [4]. The reaction mixture was stirred at room temp. for 2 hr. Excess $LiAlH_4$ was then decomposed by addition of EtOAc (1 ml). NaOH soln (5%, 1 ml) and H_2O_2 (30%, 1 ml) were then added, stirred for 1 hr, neutralised with H_2SO_4 and extracted with Et_2O. The ethereal layer was then washed with $NaHCO_3$ soln and H_2O, dried and evapd. The crude product (35 mg) was purified by chromatography over Si gel and crystallised from $CHCl_3$–EtOH, mp 236°. It was identical with gymnosporol in all respects (TLC, mmp and IR).

Compound H (wallichianic acid). Mp 280°, $[\alpha]_D$ 28° (*c* 1% $CHCl_3$ EtOH); ν_{max}^{KBr} cm^{-1}: 3400–2605, 1700 (COOH), 2940, 2860, 1450, 1370, 1035; MS: *m/e* 458 (M^+), 443 (M-15), 440 (M-18), 425 (M-18-15), 397, 385 (M-73), 367, 304, 289, 221, 207, 189, 163, 149, 135, 121, 109, 107. (Found: C, 78.75; H, 10.85. $C_{30}H_{50}O_3$ requires C, 78.54; H, 10.98%). Methylation of wallichianic acid with CH_2N_2 furnished methyl wallichianate, which crystallised from EtOH, mp 212°, $[\alpha]_D$ 21° (*c* 1%, $CHCl_3$); ν_{max}^{KBr} cm^{-1} 3360, 1040 (OH), 1720, 1185 (COOMe); PMR: δ 0.77 (6H, *s*, 2 × C-Me), 0.86, 0.93, 0.96, 1.03 (3H each, *s*, 4 × C-Me), 1.03 (3H, *d*, J = 7 Hz, C-Me), 2.75 (1H, *qd*, J = 7 and 3 Hz, Me-C<u>H</u>-COOMe, on 90 MHz machine), 3.18 (1H, *m*, C<u>H</u>OH), 3.65 (3H, *s*, COOMe); MS: *m/e* 472 (M^+), 457 (M-15), 454 (M-18), 439 (M-18-15), 423, 411, 385 (M-87), 367 (M-87-18, m* at 349.8), 352, 318, 303, 275, 249, 235, 207, 189, 177, 175, 163, 149, 141, 135, 121, 109, 107. Methyl wallichianate was acetylated by Ac_2O–C_5H_5N to give a monoacetate which crystallised from EtOH, mp 282°, $[\alpha]_D$ 31° (*c* 1%, $CHCl_3$); ν_{max}^{KBr} cm^{-1}: 1730, 1235 (OCOMe), 1720, 1175 (COOMe); PMR: δ 0.77, 0.93, 1.05 (3H each, *s*, C-Me), 0.86 (9H, *s*, 3 × C-Me), 1.03 (3H, *d*, J = 7 Hz, C-Me), 2.02 (3H, *s*, COOMe), 2.80 (1H, *qd*, J = 7 and 3 Hz, Me-C<u>H</u>-COOMe), 3.63 (3H, *s*, COOMe), 4.5 (1H, *m*, CHOAc); Me: *m/e* 514 (M), 499 (M-15), 454 (M-60), 439 (M-60-15), 427 (M-87), 411, 367 (M-87-60), 249, 235 and 189.

$LiAlH_4$ reduction of methyl wallichianate. To a soln of methyl wallichianate (100 mg) in THF (10 ml) was added $LiAlH_4$ (30 mg) and the reaction mixture was refluxed for 5 hr followed by usual work up. The product (wallichianol) was purified by chromatography and crystallised from EtOH, mp 266° (40 mg). It was identical with compound F_1 in all respects (TLC, mmp, IR). Wallichianol (28 mg) was tosylated followed by $LiAlH_4$ reduction as usual. The product (24 mg) was purified by chromatography and crystallised from EtOH, mp 204 (9 mg) and was identified as dihydrolupeol by direct comparison with the authentic sample (TLC, mmp, IR).

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