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A Thiazole and Two β-Carboline Constituents from *Panax ginseng*

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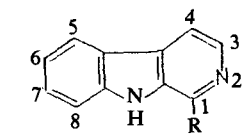
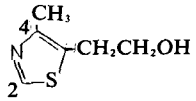
Abstract □ From the ether soluble alkaloidal fraction of *Panax ginseng*, 4-methyl-5-thiazoleethanol, norharman and harman were isolated. 4-Methyl-5-thiazoleethanol was isolated for the first time from natural resources.

Keywords □ *Panax ginseng*, Araliaceae, alkaloid, β-carboline, 4-methyl-5-thiazoleethanol, norharman, harman.

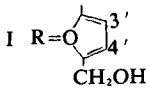
The isolation and structural determination of three β-carboline alkaloids from the root of *Panax ginseng* C.A. Meyer was reported by Han *et al.*[1] for the first time. Afterwards, we also isolated two other β-carboline alkaloids[2] and carried out the comparison of alkaloidal fractions from *Panax* genus by TLC[3]. Continuous chemical studies of the alkaloidal fraction from *Panax ginseng* have resulted in the isolation of a thiazole compound and two β-carboline alkaloids along with a known β-carboline alkaloid. In connection with these studies, we wish to report the structural elucidation of these compounds.

The ether soluble alkaloidal fraction was repeatedly chromatographed by a combination of silica gel column chromatography and preparative TLC to give compounds I, II, III and IV, and they showed positive Dragendorff reaction. UV spectra of compounds I, III and IV exhibited the typical absorption patterns of β-carboline skeleton[4], whereas compound II showed the presence of aromatic ring.





II



I R=

III R = H
IV R = CH_3

Compound II was obtained as yellowish oily liquid by semi-preparative HPLC and showed M^+ at *m/z* 143 in its mass spectrum. Its ^{1}H-NMR spectrum exhibited a methyl signal at δ2.40 (3H, s), two methylene protons of hydroxyethyl group at δ 3.02 (2H, t, J=7.1 Hz) and 3.84 (2H, 5, J=7.1 Hz), and an olefinic proton[5] due to thiazole ring at δ 8.61 (1H, s), suggesting II to be 4-methyl-5-thiazoleethanol. This was supported by its mass fragment ions, *m/z* 125 (M^+-H_2O), 112 (M^+-CH_2OH) and 85 (M^+-CH_2CH_2OH-CH_3), characteristic ion of thiazole ring[6]. Compound II was conformed as 4-methyl-5-thiazoleethanol by direct comparison with its standard ^{1}H-NMR spectral data[7]. This compound has been used as an intermediate, the thiazole moiety of thiamine, for synthesis of thiamine. To our best knowledge, it was the first report for the isolation of this compound from natural resources.

Compound III was recrystallized from benzene to give palely yellowish needle, mp 192° and showed M^+ at *m/z* 168 in its mass spectrum. Its IR spectrum exhibited absorption band due to amine (3400 cm^{-1}) group. ^{1}H-NMR spectrum of this compound showed seven protons assignable to β-carboline at δ 7.14-8.87 along with a proton signal of C-1 position at δ 8.87 (1H, s), indicating that it is unsubstituted β-carboline, norharman. This was further supported by its mass fragment ions, *m/z* 141, 114 corresponding to the consecutive loss of HCN from molecular ion peak[8]. Acetylation of this compound provided 1-acetyl-β-carboline, suggesting C_1-acetylation of compound III. Consequently, compound III was identified as norharman, which was first isolated from ginseng. This alkaloid has already been isolated from *Codonopsis lanceolata*[9] and *Polygala tenuifolia*[10].

Compound IV was recrystallized from methylene chloride-hexane to yield palely yellowish needle, mp 184°. Its IR spectrum was similar to that of compound III and ^{1}H-NMR spectrum exhibited one methyl group at δ 2.79 (3H, s) together with aromatic protons assignable to β-carboline at δ 7.17-8.34, suggesting that one methyl group was attached to C_1. Its mass fragment ions showed *m/z*

167, corresponding to the elimination of methyl group from molecular ion peak as well as m/z 140, 113, characteristic of β-carboline[8]. On the basis of these spectral data, compound IV was identified as harman, 1-methyl-β-carboline, which was first isolated from ginseng. This alkaloid was previously isolated from *Polygala tenuifolia*[10] and *Ochrosia nakaina*[11]. Particularly, compounds III and IV have been reported to act as one of inhibitors of monoamine oxidase[12] and to inhibit the binding of all benzo[a]pyren metabolites to DNA[13].

Compound I was recrystallized from $CHCl_3$ to give brown needle, mp 168°, and showed M^+ at m/z 264 in its mass spectrum. Its IR spectrum exhibited bands due to amine and hydroxyl (3375 cm^{-1}) groups. The ^{1}H-NMR spectrum showed aromatic protons assignable to a β-carboline at δ 7.20-8.27, and also did one methylene signal at δ 4.75 (2H, s) and two doublet peaks at δ 6.44 (1H, d), 7.16 (1H, d) with coupling constant J = 3.5 Hz, suggesting the presence of 5-hydroxymethyl-2-furyl group. This was further supported by its mass fragment ions at m/z 167 corresponding to the elimination of 5-hydroxymethyl-2-furyl group from M^+. And also acetylation of I afforded a monoacetate. From the above results, compound I was identified as 1-(5-hydroxymethyl-2-furyl)-β-carboline, which has already been isolated from ginseng[1], *Lolium perenne*[14], *Codonopsis lanceolata*[9] and *Lycium chinense*[15].

EXPERIMENTAL METHODS

The melting points were determined on a Gallenkamp melting point apparatus. IR-spectra were recorded in KBr disc by a Perkin Elmer 599B spectrometer. UV spectra were run with a Shimadzu Model UV-200S double beam spectrophotometer. ^{1}H-NMR spectra were taken on a Varian FT-80A spectrometer, operated at 80 MHz in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard and chemical shift values are quoted in ppm(δ). Mass spectra were taken on a Varian Model MAT 212 GC/MS system equipped with direct inlet system, operating at 70eV. Semi-preparative HPLC was carried out on Waters model ALC-244 using μ-Bondapak C_{18} (7.8 mm × 30 mm). Column chromatography was carried out on silica gel (Merck Art. 9385, 230-400 mesh). TLC and preparative-TLC were performed on precoated silica gel $60F_{254}$ plate (Merck) and detected by Dragendorff's reagent or by UV illumination.

Extraction and fractionation

This was carried out as described previously[2].

Isolation of alkaloids

The alkaloid fraction (2.5g) was applied to silica gel column (3 × 30cm) and eluted with chloroform, which was mixed with methanol (The mixing ratio was 100:1 (fr.I), 70: 1 (fr.II), 50:1 (fr.III), 30:1 (fr. IV) and 10:1 (fr.V), respectively.) Fr. III (254mg) was separated into subfractions III-1, III-2 and III-3. Fr. III-2 was further purified by preparative-TLC on silica gel with chloroform-ethylacetate-methanol (50:10:5) to give compound I (32mg, brown needle), which was recrystallized from chloroform. Compound II (3.8mg, yellowish oily liquid) was purified from fr. III-3, using preparative-TLC with benzene-ethylacetate (1:1) and semi-preparative HPLC (column: μ-Bondapak C_{18}, 7.8mm × 30cm) with MeOH-H_2O (80:20). Fr. IV was subjected to column chromatography on silica gel with a solvent, $CHCl_3$-MeOH (20:1). This eluate was separated into two fractions IV-1 and IV-2. The IV-2 fraction (82mg) was purified by preparative-TLC on silica gel with EtOAc/HAc/H_2O (25:1:8, upper layer) and separated again into IV-2-1 and IV-2-2. Compound III (12mg) was recrystallized with benzene from fr. IV-2-1 after basified with c-NH_4OH. Compound IV (7.2mg, yellowish needle) was recrystallized with CH_2Cl_2-hexane (1:1) from fr.IV-2-2 through the above same process by preparative-TLC.

Compound I

mp: 168°; UV λ_{max} in MeOH (log ε): 210 (4.52), 236 (4.40), 252 (4.33), 273 (4.32), 290 (4.38), 300 (4.26), 367 (4.29), 378 (4.30); IR (cm^{-1}): 3375 (-NH, -OH), 1628 (aromatic C = C), 1567 (C = N), 1490 (indole C = C), 1420, 1315, 1290; MS m/z (rel. int. %): 264 (M^+, 100), 247 (M^+-OH, 63.4), 246 (M^+-H_2O, 44.6), 235 (11.6), 233 (M^+-CH_2OH, 8.0), 218 (19.6), 205 (M^+-CH_2OH-CO, 23.2), 184 (8.5), 167 (M^+-$C_5H_5O_2$, 30.4), 140 (20.5), 114 (7.1); ^{1}H-NMR: 9.42 (1H, br. s, NH, exchanged with D_2O), 8.27 (1H,d, J = 5.3 Hz, C_3-H), 8.05 (1H,d, J = 8 Hz, C_5-H), 7.78 (1H,d, J = 5.2 Hz, C_4-H), 7.58-7.40 (2H, m, $C_{6,8}$-H), 7.35-7.20 (1H, m, C_7-H), 7.16(1H,d, J=3.5 Hz, $C_{4'}$-H), 6.44 (^{1}H,d, J = 3.5 Hz, $C_{3'}$-H), 4.75 (2H,s,-$\underline{CH_2}$OH)

Acetylation of I

Fifteen mg of VI in pyridine (2m*l*) and Ac_2O (2m*l*) was allowed to stand at room temperature overnight, and followed by the usual work. The residue was crystallized from EtOAc to give palely brown needle (8mg). mp: 162°; ^{1}H-NMR: 9.82 (1H, br.s, NH, exchanged with D_2O), 8.37 (1H,d, J = 5.3 Hz,C_3-H), 8.08 (1H,d, J = 7.5 Hz, C_5-H), 7.82 (1H,d, J = 5.3 Hz, C_4-H), 7.61-7.43 (2H, m, $C_{6,8}$-H), 7.35-7.26 (1H, m, C_7-H), 7.18 (1H,d,

J = 3.6 Hz, C_4-H), 6.55 (1H,d, J = 3.6 Hz, C_3-H), 5.22 (2H, s,-$\underline{CH_2}OCOCH_3$), 2.11 (3H,s, $CH_2OCO\underline{CH_3}$); MS *m/z* (rel. int. %): 306 (M^+, 64.9), 263 (M^+-$COCH_3$, 17.5), 246 (M^+-$COCH_3$-OH, 100), 218 (19.6), 205 (M^+-CH_2OCOCH_3-CO, 10.5), 167 (24.6), 140 (13.2), 114 (3.5)

Compound II

Palely yellow oily liquid. UV λ_{max} in MeOH (log ε): 212 (4.64), 252 (4.48); MS *m/z* (rel. int. %): 143 (M^+, 41.5), 125 (M^+-H_2O, 3.6), 112 (M^+-CH_2OH, 100), 85 (M^+-CH_3-CH_2CH_2OH + 2H, 33.6), 71 (7.9), 59 (15.9); ^{1}H-NMR: 8.61 (1H,s, C_2-H), 3.84 (2H,t, J = 7.1 Hz,-$\underline{CH_2}CH_2OH$), 3.02 (2H,t, J = 7.1 Hz,-$CH_2\underline{CH_2}OH$), 2.40 (3H, s,-CH_3)

Compound III

mp: 192°; UVλ_{max} in MeOH (log ε): 210 (4.09), 234 (4.46), 250 (4.28), 282 (4.03), 287 (4.20), 334 (3.68), 350 (3.66); IR (cm^{-1}): 3400(NH), 1625 (aromatic C = C), 1560 (C = N), 1450, 1330, 1282; MS *m/z* (rel. int. %): 168 (M^+, 100), 141 (M^+-HCN, 9.5), 140 (5.6), 128 (1.7), 114 (M^+-HCN-HCN, 9.1), 113 (6.0), 84 (11.3), 70 (7.8); ^{1}H-NMR: 8.92 (1H, br. s, HN, exchanged with D_2O), 8.87 (1H,s, C_1-H), 8.42 (1H,d, J = 5 Hz, C_3-H), 8.13 (1H,d, J = 7.5 Hz, C_5-H), 7.94 (1H,d, J = 5 Hz, C_4-H), 7.53-7.46 (2H, m, $C_{6,8}$-H), 7.34-7.14 (1H,m, C_7-H)

Acetylation of III

Six mg of III in pyridine (1m*l*) and Ac_2O (1m*l*) was allowed to stand at room temperature overnight, and followed by the usual work. The resiude was crystallized from benzene to give palely yellowish needle (3mg). mp: 201-203°; ^{1}H-NMR: 9.58 (1H, br.s, NH, exchanged with D_2O), 8.59 (1H,d, J = 5.4 Hz, C_3-H), 8.21 (1H,d, J = 8 Hz, C_5-H), 8.04 (1H,dd, J = 8 Hz, C_8-H), 7.85 (1H,d, J = 5.4 Hz, C_4-H), 7.68-7.37 (2H,m, $C_{6,7}$-H), 2.91 (3H, s,-$COCH_3$)

Compound IV

mp: 184°; UVλ_{max} in MeOH (log ε): 234 (4.48), 242 (4.51), 252 (4.23), 282 (4.08), 287 (4.38), 334 (3.73), 347 (3.77); IR (cm^{-1}): 3400(-HN), 1623 (aromatic C = C), 1567 (C = N), 1500, 1480 (indole C = C); MS *m/z* (rel. int. %): 182 (M^+, 100), 167 (M^+-CH_3, 1.2), 154 (19.5), 141 (1.3), 140 (M^+-CH_3-HCN, 4.2), 127 (7.9), 114 (3.1), 113 (M^+-CH_3-HCN-HCN, 1.2); ^{1}H-NMR: 8.88 (1H, br.s, exchanged with D_2O), 8.34 (1H,d, J = 5 Hz, C_3-H), 8.08 (1H,d, J = 7.5 Hz, C_5-H), 7.78 (1H,d, J = 5 Hz, C_4-H), 7.52-7.46 (2H,m, $C_{6,8}$-H), 7.34-7.17 (1H,m, C_7-H), 2.79 (3H, s,-CH_3)

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