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Note

Chemical constituents of *Taxus canadensis*

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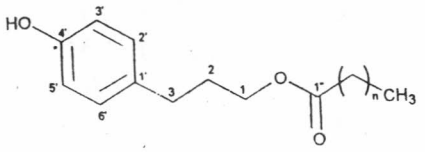
Phytochemical investigation of *Taxus canadensis* leads to the identification of four new esters, namely 3-(4-hydroxyphenyl)propyl pentacosanoate **1**, 3-(4-hydroxyphenyl)propyl hexacosanoate **2**, 3-(4-hydroxyphenyl)propyl dotriacontanoate **3** and 3-(4-hydroxyphenyl)-propyl tetratriacontanoate **4** along with one known ester, 3-(4-hydroxyphenyl)propyl tetracosanoate **5**. Six additional compounds, sciadopitysin **6**, ginkgetin **7**, rhododendrin **8**, taxicatin **9**, taxinine **10** and β-sitosterol have also been isolated and identified.

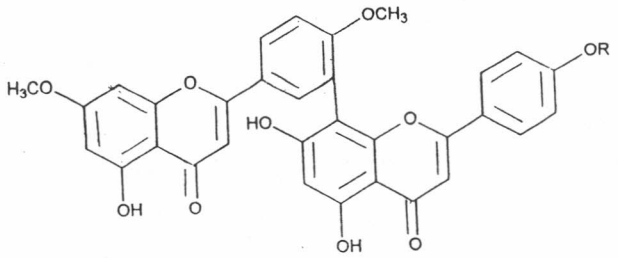
The genus *Taxus* belongs to the family Taxaceae and its various species are of significance as they have been found to possess a number of biological activities such as cytotoxic, antileukemic, sedative, antiseptic, tranquilising and antimitotic. Earlier phytochemical work on *Taxus canadensis* has resulted in the isolation of ten different taxanes[1-5]. In the present investigation of the petroleum ether and ethanol extracts of the leaves and twigs of *Taxus canadensis*, four new fatty acid esters, namely 3-(4-hydroxyphenyl)propyl pentacosanoate **1**, 3-(4-hydroxyphenyl)propyl hexacosanoate **2**, 3-(4-hydroxyphenyl)propyl dotriacontanoate **3** and 3-(4-hydroxyphenyl)propyl tetratriacontanoate **4** have been isolated and characterized, in addition to 3-(4-hydroxyphenyl)propyl tetracosanoate **5** (earlier known only once, i.e., from *Piper clarkii*[6]), sciadopitysin **6**, ginkgetin **7**, rhododendrin **8**, taxicatin **9**, taxinine **10** and β-sitosterol. Of these compounds only taxinine has been reported earlier from this plant.

The dried leaves and twigs of *Taxus canadensis* were exhaustively extracted with hot petroleum ether and ethanol in succession. The concentrate of the petroleum ether extract was column chromatographed over silica gel, and elution with petroleum ether-chloroform gave β-sitosterol. Further elution with petroleum ether-chloroform furnished two waxy solids, named mixture 'A' and mixture 'B'. The IR and ^{1}H NMR spectra of these solids were quite similar. The IR spectra of both substrances showed the presence of a phenolic OH (3300 cm^{-1}) and an ester moiety (1720 and 1142 cm^{-1}). The ^{1}H NMR spectra exhibited signals for an aliphatic methyl (δ 0.88), a $-CH_2CH_2CH_2-$ moiety (δ 4.08, 2.61 and 1.91) attached to electron-withdrawing groups on both sides, a methylene α to a carbonyl group (δ 2.30), another methylene β-to a carbonyl group (δ 1.62) and a broad singlet at δ 1.26. The presence of a $-CH_2CH_2CH_2-$ moiety was further confirmed by the expected cross peaks in the ^{1}H-^{1}H COSY NMR spectra of 'A' and 'B'. The peaks in the aromatic region showed a pattern typical for a *para*-disubstituted benzene ring.

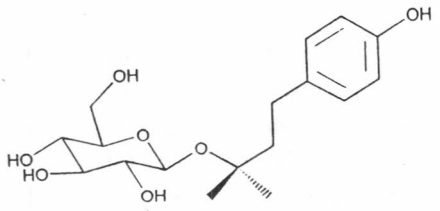
The CI mass spectra of mixture 'A' exhibited, as only significant ions, *pseudo* molecular ions $[M+H]^+$ corresponding to three major components **5, 1** and **2** with M_r values of 502, 516 and 530. Integration of ion traces *m/z* 503, 517 and 531 over the evaporation profile indicated that the three compounds were present in an approximate ratio of 5:1:2. Trace amounts of homologs (M_r 474, 488, 544, 558, 586, 614) were also present. In the EI mass spectra, intense signals common to all compounds were present at *m/z* 107 ($[HO-Ph-CH_2]^+$) and 134 ($[HO-Ph-CH_2CH=CH_2]^+$), the latter being formed by β-cleavage at the ester bond. In addition, peaks due to α- and β-cleavage (with McLafferty rearrangement) at the ester bond and charge retention by the carboxylic acid moiety were observed for all the three major components. For **5**, these peaks were observed at *m/z* 351 and 368, for **1** at *m/z* 365 and 382 and for **2** at *m/z* 379 and 396. The presence of **5** and **1** was confirmed by GC/MS of the silylated mixture. The sample was first derivatized (as TMS derivative) using bis silyltrifluoroacetamide and pyridine in acetonitrile. Total ion current (TIC) extraction of TMS



1 n=23
2 n=24
3 n=30
4 n=32
5 n=22



6 R=CH_3
7 R=H



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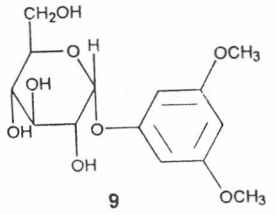
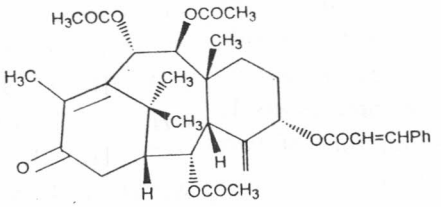


9



10

derivatived mixture-A suggested that scan numbers 245 and 427 contain appreciable ion current. The mass spectrum of the ions extracted at around scan numbers 245 and 427 showed molecular ion peaks at *m/z* 574 and 588, respectively. In addition both showed an intense ion at *m/z* 206 ([TMC–O–Ph–$CH_2CH{=}CH_2$]$^+$) due to β-cleavage at the ester bond. The solid 'A' was thus identified as an inseparable mixture of 3-(4-hydroxyphenyl)propyl pentacosanoate **1**, 3-(4-hydroxyphenyl)propyl hexacosanoate **2** and 3-(4-hydroxyphenyl)propyl tetracosanoate **5** in the ratio 1:2:5.

The IR, ^{1}H and ^{13}C NMR spectra of mixture-B were very similar to those of mixture-A suggesting the same basic structure for their components. The CI mass spectra showed major $[M+H]^+$ peaks corresponding to compounds **3** and **4** with M_r values of 614 and 642, respectively, in an approximate ratio of 2:1. The EI mass spectra confirmed the molecular weights. Other homologs (M_r 558, 586, 600, 628) were also present in trace amounts. The solid 'B' was thus identified as an inseparable mixture of 3-(4-hydroxyphenyl)propyl dotriacontanoate **3** and 3-(4-hydroxyphenyl)propyl tetratriacontanoate **4** in the ratio of 2:1.

The ethanolic extract of *Taxus canadensis* on column chromatography yielded five crystalline compounds **6-10**. Their physical constants and spectral data were in good agreement with those reported for sciadopitysin **6**[7,8], ginkgetin **7**[7,8], rhododendrin **8**[9], taxicatin **9**[10] and taxinine **10**[11-14], respectively.

Experimental Section

General. All melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 250P spectrometer at 250 and 62.9MHz, respectively (chemical shifts in δ, ppm relative to TMS). EIMS and CIMS were recorded on a Jeol AX505W mass spectrometer at 70 eV. GC-MS was performed on a VG Trio I using a 30m DB-1 capillary column at 250°C.

Extraction and isolation. The leaves and twigs of *Taxus canadensis* were procurred from Odense University Campus, Denmark, in January 1992. The dried plant material (1 kg) was extracted exhaustively with petroleum ether and ethanol in succession in a soxhlet apparatus. Both extracts were concentrated below 50°C under reduced pressure.

Column chromatography of the petroleum ether extract (25g) was carried out on a silica gel column. The fractions eluted with petrol-chloroform (1:1, 1:4 and 3:7) mixtures yielded β-sitosterol, an inseparable mixture of three esters **1, 2** and **5** (mixture-A) and another mixture of two esters **3** and **4** (mixture-B), respectively.

Column chromatography of the ethanol extract (150g) on elution with chloroform-methanol (9:1) gave sciadopitysin **6**, ginkgetin **7** and taxinine **10** and the chloroform-methanol (7:3) eluates yielded taxicatin **9**. Rhododendrin **8** was obtained as a major component (4% by weight of the ethanolic extract) from the chloroform-methanol (3:2) eluates.

Mixture-A (compounds 1, 2 and 5). White waxy solid (15mg); UV (MeOH): 226, 280nm; UV (MeOH+NaOMe): 226, 243 (sh), 281, 302 (sh) nm; IR (nujol): 3300, 1720, 1240, 1142 cm^{-1}; EIMS (relative intensity): *m/z* 107 $[HO–Ph–CH_2]^+$ (24), 134 $[HO–Ph–CH_2CH=CH_2]^+$ (100), 351, 365, 368, 379, 382, 396, 502, 516, 530, 544, 558, 586, 614 (all less than 3% along evaporation profile); CIMS (relative intensity): *m/z* 475, 489, 503, 517, 531, 545, 559, 587, 615 (intensities vary along evaporation profile); ^1H NMR ($CDCl_3$): δ0.88 (t, *J*=7Hz, 3H, $–CH_3$), 1.26 (brs, C-4′′H to C-23′′H/C-24′′H/C-25′′H), 1.62 (brm, 2H, $–OCOCH_2CH_2$), 1.91 (m, 2H, $–COOCH_2CH_2$), 2.30 (t, *J*=7.5 Hz, 2H, $–OCOCH_2–$), 2.61 (t, *J*=7.6Hz, 2H, $ArCH_2–$), 4.08 (t, *J*=7Hz, 2H, $–COOCH_2–$ 6.75 (d, *J*=10Hz, 2H, C-3′H and C-5′H), 7.03 (d, *J*=10Hz, 2H, C-2′H and C-6′H); ^{13}C NMR ($CDCl_3$): δ14.11 (C-24′′/C-25′′/C-26′′), 22.68 (C-23′′/C-24′′/C-25′′), 25.03 (C-3′′), 29.18-29.69 (C-5′′ to C-22′′/C-23′′/C-24′′), 30.46 (C-4′′), 31.25 (C-2), 31.92 (C-2′′), 34.39 (C-3), 63.54 (C-1), 115.25 (C-3′ and C-5′), 129.38 (C-2′ and C-6′), 133.34 (C-1′), 153.84 (C-4′), 174.01 (C-1′′).

Mixture-B (compounds 3 and 4). White solid (20 mg), mp 80-84°C; UV (MeOH): 224, 276nm; UV (MeOH+NaOMe): 216, 240 (sh), 280, 296 (sh) nm; IR (nujol): 3300, 2850, 1725, 1505, 1240, 1160, 1000 cm^{-1}; EIMS (relative intensity): *m/z* 107 $[HO–Ph–CH_2]^+$ (21), 134 $[HO–Ph–CH_2CH=CH_2]^+$ (100), 463, 480, 491, 508, 586, 614, 628, 642 (all less than 3% along evaporation profile); CIMS (relative intensity): *m/z* 531, 545, 559, 573, 587, 601, 615, 629, 643, 657 (intensities vary along evaporation profile); ^1H NMR ($CDCl_3$): δ0.88 (t, *J*=7Hz, 3H, $–CH_3$), 1.26 (brs, C-4′′H to C-31′′HC-33′′H), 1.62 (brm, 2H, $–OCOCH_2-CH_2–$), 1.91 (m, 2H, $–COOCH_2CH_2–$), 2.30 (t, *J*=7.5 Hz, 2H, $–COCH_2–$), 2.61 (t, *J*=7.6Hz, 2H, $ArCH_2–$), 4.08 (t, *J*=7Hz, 2H, $–COOCH_2–$), 4.70 (1H, brs, –OH), 6.75 (d, *J*=10Hz, 2H, C-3′H and C-5′H), 7.03 (d, *J*=10Hz, 2H, C-2′H and C-6′H); ^{13}C NMR ($CDCl_3$): δ14.11 (C-32′′/C-34′′), 22.68 (C-31′′/ C-33′′), 25.03 (C-3′′), 29.18-29.70 (C-5′′ to C-30′′/C-32′′), 30.46 (C-4′′), 31.25 (C-2), 31.93 (C-2′′), 34.39 (C-3), 63.54 (C-1), 115.23 (C-3′ and C-5′), 129.47 (C-2′ and C-6′), 133.40 (C-1′), 153.78 (C-4′), 174.03 (C-1′′).

Compound 6. Greenish yellow powder (25 mg), mp 297-300°C (lit.[7] mp 315°C, lit.[8] mp 290°C). All the spectral data were identical with those of sciadopitysin[7,8].

Compound 7. Greenish yellow powder (20mg), mp >300°C (lit.[7] mp 320°C, lit.[8] mp 327°C). All the spectral data were in good agreement with the values reported for ginkgetin[7,8].

Compound 8. Shiny colourless cubes (6.5g), mp 188-190°C (lit.[9] mp 189-191°C). All the spectral data tallied well with those reported for rhododendrin in literature[9].

Compound 9. Cream coloured crystalline solid (200 mg), mp 173-175°C (lit.[10] mp 170-71°C). The spectral data matched well with the data reported for taxicatin[10].

Compound 10. Light green crystalline solid (60mg), mp 261-262°C [lit.[11] mp 264-265°C]; $[\alpha]_D^{24}$+126.8° (*c* 0.78, MeOH), lit.[12] $[\alpha]_D^{18}$+ 137°($CHCl_3$). All these spectral data are identical with those for taxinine **10**[11-14].

Compound 11. Colourless needles (80mg), mp 136°C. The mixed melting point with an authentic sample of β-sitosterol remained underpressed. Co-TLC with an authentic sample of β-sitosterol and superimposable IR spectra confirmed its identity.

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