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CASTANOPSONE AND CASTANOPSOL TWO NEW TRITERPENOIDS FROM *CASTANOPSIS INDICA**

PUSHPA PANT and R. P. RASTOGI
Central Drug Research Institute, Lucknow, India

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Key Word Index—*Castanopsis indica*; Fagaceae; psoralen; angelicin; triterpenoids; erythrodiol; ursolic acid; castanopsin; castanopsone; castanopsol; sitosterol.

Abstract—The isolation of two coumarins and five triterpenoids from *Castanopsis indica* is described. These have not been reported from this genus previously. The structures of two of the three new triterpenoids have been elucidated by chemical reactions and physical methods.

INTRODUCTION

Castanopsis indica (Fagaceae) a medium sized tree, is commonly distributed over tropical Himalayas, Assam and Khasi hill up to 1300 m. Various species of *Castanopsis* such as *C. cuspidata, C. concinna, C. eyrei, C. fabri, C. hickelii* and *C. fissa* have been investigated and neutral triterpenoids e.g., friedelin, friedelane-3-ol, its 3α-yl acetate, canophyllol, glutionol, taraxerol, taraxsterol, lupeol, betulin, 22-hydroxy-hopan-3-one, hopan-3β,22-diol have been isolated [1–3]. The stem bark of *C. indica* was found to exhibit anticancer activity in KB (ED_{50}, 2.2 μg/ml) and PS systems [4] and, therefore, a chemical examination has been undertaken resulting in the isolation of eight substances, two of which are coumarins. Amongst the triterpenoids, three were found to be new products and the elucidation of the structures of two of these, namely castanopsone and castanopsol, is now described.

RESULTS AND DISCUSSION

The ethanolic extract of the stem bark was successively fractionated into hexane, $CHCl_3$ and BuOH soluble fractions. The hexane and $CHCl_3$ fractions showed an identical ten spots on TLC which were designated as substances, A, B, C, D, E, F, G (castanopsone), H (castanopsin), I and J (castanopsol) in decreasing order of R_f values.

The lipid fraction was subjected to chromatographic separation. The hexane–C_6H_6 eluate containing substance A, was oily and was not worked up because of insufficient quantity. The C_6H_6–$CHCl_3$ eluates gave a mixture of substances B, C, D and E from which the individual components were separated by rechromatography over neutral alumina. The $CHCl_3$ and $CHCl_3$–EtOAc elautes were a mixture of substances F, G, H and I and repeated chromatography over Si gel using hexane with increasing proportions of ether and finally $CHCl_3$, led to the isolation of pure substances. Substance J was isolated by rechromatography of the tailend $CHCl_3$–EtOAc eluate using hexane–Et_2O on $AgNO_3$ impregnated Si gel.

The BuOH soluble fraction as well as its Si gel and polyamide column eluates showed only extensive streaking on TLC and no constituent could be isolated from it. The biological activity was found to be located in the H_2O-soluble fraction of the plant extract, which was chromatographed over polyamide. The H_2O and H_2O–MeOH (1:1) eluates showed activity (T/C 131 %, PS) and these are now under investigation.

Castanopsone, mp 145°, $C_{30}H_{48}O_2$ (M^+440), gave a violet colour in the Liebermann Burchard test and a yellow colour with tetranitromethane which indicated it to be an unsaturated triterpenoid. The IR absorptions at 3475, 1705 and 805 cm^{-1} indicated the presence of hydroxyl, six membered ring carbonyl and a trisubstituted double bond in the molecule. Its NMR spectrum showed eight tertiary methyl singlets at δ 0.78, 0.83, 0.96, 1.10 ppm a carbinol proton at 3.50 ppm and an olefinic proton multiplet at 5.21 ppm.

It gave a monoacetate (**1b**) whose NMR spectrum exhibited an acetoxymethyl signal at δ 2.03 ppm and the carbinolic proton appeared at 4.81 ppm as a quartet showing a downfield shift of 1.3 ppm which confirmed the presence of a secondary hydroxyl group in the molecule. The formation of a dinitrophenyl hydrazone and production of a diol (**1c**) on $NaBH_4$ reduction confirmed the presence of a six membered ring ketone. An attempt to reduce the double bond catalytically, led to a rather unusual reduction of the carbonyl group and gave the same diol in almost quantitative yield.

Its MS showed peaks at *m/e* 218 (base peak), 203, 189, 190 and 177 arising from retro-Diels Alder fragmentation which are characteristic for olean-12-ene [5]. The fragmentation ions showed that the allocation of the hydroxyl and keto groups was restricted only to rings A and B. An attempt to prepare deoxycastanospsone by the Wolff–Kishner method did not succeed but resulted in the formation of olean-2,12-dien-1-one (**2**) [6] by the elimination of secondary hydroxyl group as shown by its IR band at 1680 cm^{-1}, the UV maximum at 232 nm and a pair of one proton doublets at 5.67 ppm and 6.36

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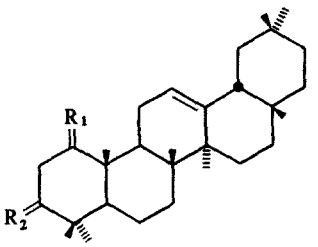
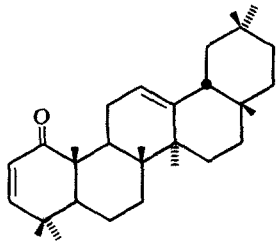


1a, $R_1 = =O$ $R_2 = \alpha$-OH, β-H

1b, $R_1 = =O$ $R_2 = \alpha$-OAc, β-H

1c, $R_1 = \alpha$-H, β-HO $R_2 = \alpha$-OH, β-H

1d, $R_1 = R_2 = =O$



2

ppm (J = 10 Hz) in the NMR spectrum which has been shown to be characteristic for Δ^2 olefinic protons [7]. The same compound was obtained by the elimination of the OH group through mesylation [8]. Oxidation of castanopsone with pyridinium chlorochromate gave castanopsdione, the UV maximum at 259 nm showed a bathochromic shift of 30 nm in presence of NaOH and the IR absorption at 1729 and 1700 cm^{-1} indicated that it was a β-diketo-derivative (**1d**) which was identified as olean-12-ene-1,3-dione [6, 9]. The CD curve of castanopsone showed θ_{286} + 1419° and its Δ^2 derivative showed θ_{337} − 7029°. The CD data for C-1 ketones of pentacyclic triterpenes is not well documented but from the values of Δ^1 and Δ^2 ketones [7, 10], it is obvious that Δ^2-1-one gives a value of higher order.

The stereochemistry of the hydroxyl group at C-3 was deduced from the NMR data of castanopsone acetate. The methine proton geminal to the acetyl group gave rise to a quartet centered at 4.81 ppm compatible with its axial position [11]; the OH group would, therefore, have the β (eq) configuration and the structure of castanopsone was elucidated as 3β-hydroxyolean-12-ene-1-one (**1a**).

Castanopsol, mp 204–5°, analysed for $C_{30}H_{50}O_2$ (M^+442) and showed the positive colour reactions of an unsaturated triterpenoid. The IR bands at 3380, 1040 and 810 cm^{-1} indicated the presence of OH and a trisubstituted double bond in the molecule. The NMR spectrum exhibited eight tertiary methyls in the region of δ0.80–1.10 ppm, two carbinolic protons as a multiplet centered at 3.7 ppm and an olefinic proton at 5.2 ppm. Castanopsol gave a diacetate whose NMR spectrum showed two acetoxymethyl singlets at 1.98 and 2.06 ppm and the two methine protons on the carbon bearing oxygen at 4.8 ppm which confirmed the presence of two secondary hydroxyls were fixed as C-1 and C-3.
the hydroxyl groups were confined only to rings A and B and its oxidation with pyridinium chlorochromate gave a diketo derivative whose physical data was identical to castanopsdione. Consequently, the positions of the secondary hydroxyls were fixed as C-1 and C-3.

The stereochemistry of the OH groups was deduced from the NMR spectrum of castanopsol diacetate. The two carbinolic protons gave rise to a partly overlapped quartet and triplet with a total width of 23 Hz. The J_{aa} and J_{ae} values of the quartet being 10 and 6 Hz respectively whereas the $W_{\frac{1}{2}}$ of the triplet was of the order of 7 Hz. This was in agreement with one α(ax) OH and the other β(eq)OH group. Since the diol (**1c**) from castanopsone and castanopsol were found to be identical, C-3 OH was β(eq) and C-1 OH was assigned the α(ax) configuration. This was also confirmed by the NMR spectrum of the $NaBH_4$ reduction product of castanopsone acetate in which the methine proton geminal to the OH group appeared as a triplet with $W_{\frac{1}{2}}$ = 7 Hz and the corresponding proton geminal to the acetyl group appeared as a quartet. Thus, the structure of castanopsol was olean-12-ene-1α,3β-diol (**1c**).

EXPERIMENTAL

Mp's are uncorr. NMR spectra were recorded at 60 MHz in $CDCl_3$ with TMS as internal standard. R_f values refer to Si gel plates using spray reagents $FeCl_3$–$K_3Fe(CN)_6$ for substances B and C and 1% ceric sulphate in 2N H_2SO_4 for the others. The plant material was collected in December 1969 from Sukna, Darjeeling District, West Bengal and identified by Mr. K. K. Singh. A voucher specimen of the plant (No. 3126/2555) is kept in the herbarium of the Institute. The ethanolic extract of the powdered stem bark (10 kg) was macerated successively with hexane, $CHCl_3$ and BuOH. The hexane fraction (56.5 g) was partitioned between hexane and 80% MeOH; the lipid fraction (47 g) was chromatographed over Si gel (220 g) and C_6H_6–$CHCl_3$ (3:1) and $CHCl_3$ and $CHCl_3$–EtOAc eluates were collected. The former eluate (4.1 g) was rechromatographed over neutral alumina (125 g) in hexane with progressively increasing proportions of C_6H_6 and finally EtOAc. Twenty two fractions (200 ml each) were collected and screened for various components by TLC developed with C_6H_6–MeOH (99:1). Substances B (0.25 g) and C (0.20 g) were isolated from hexane–C_6H_6 (1:1), substance D (0.32 g) from C_6H_6 and substance E (0.5 g) from EtOAc eluates. The $CHCl_3$–EtOAc (3:1) (16.0 g) fraction was repeatedly chromatographed over Si gel and substance F (0.20 g) was obtained from hexane–Et_2O (3:1); substance G (1.21 g) from hexane–Et_2O (2:1); substance H (0.30 g) from hexane–Et_2O (1:1) and substance I (0.15 g) from $CHCl_3$ fractions. Substance J was isolated by subsequent chromatography of the $CHCl_3$–EtOAc (1:1) eluate over $AgNO_3$ impregnated Si gel using hexane–Et_2O (1:2) as eluant.

Substance B (psoralen). Colourless flakes from $CHCl_3$–hexane, mp 161–62°; R_f 0.71 (C_6H_6–MeOH, 99:1). λ_{max}^{EtOH} nm (log ε): 247, 293 (3.40, 3.05). ν_{max}^{KBr} cm^{-1}: 3160–3060, 1760, 1714 (α pyrone), 1667, 1577 (aromatic) 1055, 834, 753 (benzofuran). NMR: δ 6.3 (1H, *d*, J = 10 Hz, H-3), 6.8 (1H, *br s*, H-3'), 7.45 (1H, *br s*, H-2'), 7.68 (2H, *s*, H-5, 8). 7.78 (1H, *d*, J = 10 Hz, H-4). MS: *m/e* 186 (M^+).

Substance C (angelicin). Colourless needles from $CHCl_3$–hexane, mp 134–35° R_f 0.60 (C_6H_6–MeOH, 99:1) λ_{max}^{EtOH} log ε:

296, 246 nm (3.92, 4.35). ν_{max}^{KBr} cm^{-1}: 2920, 1735, 1710 (αpyrone), 1615, 1535 (aromatic), 1055, 830, 747 (benzofuran). NMR: δ 6.35 (1H, *d*, *J* = 10 Hz, H-3), 7.08 (1H, *d*, *J* = 2 Hz, H-3'), 7.3 (2H, *s*, H-5, 6), 7.68 (1H, *d*, *J* = 2 Hz, H-2'), 7.76 (1 H, *d*, *J* = 10 Hz, H-4). MS: *m/e* 186 (M^+).

Substance E (sitosterol). Colourless flakes from alcohol, mp 136°; R_f 0.45 (C_6H_6–MeOH, 99:1), $[\alpha]_D$ − 35° (*c* 1.0, $CHCl_3$). Acetate, mp 125°.

Substance F (erythrodiol). Colourless needles, mp 238–42°, $[\alpha]_D$ + 75°, R_f 0.45 ($CHCl_3$–MeOH, 49:1). ν_{max}^{KBr} cm^{-1}: 3370, 1095, 1040 (OH), 2920, 2870, 1385, 1370, 835. NMR: δ 0.72, (3H, *s*, Me), 0.84 (6H, *s*, 2 × Me), 0.91 (9H, *s*, 3 × Me), 1.12 (3H, *s*, Me), 3.32 (3H, *m*, —C<u>H</u>$_2$OH and —C<u>H</u>OH), 5.32 (1H, *m*, —C=C<u>H</u>—). MS: *m/e* 442 (M^+), 427, 412, 353, 234 (base peak), 221, 220, 207, 203, 191, 190, 189, 177, 149, 133. (Found: C, 81.39; H, 11.35 $C_{30}H_{50}O_2$ requires C, 81.44; H, 11.31%). Diacetate, mp 180°. MS: (*m/e*) 526 (M^+).

Substance G (castanopsone). Colourless needles from MeCN, mp 145°, R_f 0.42 ($CHCl_3$–MeOH, 49:1). $[\alpha]_D$ + 128.1° (*c*, 1.0, $CHCl_3$) ν_{max}^{KBr} cm^{-1}: 3475, 1040 (OH), 2940, 2870, 1705, 1695 (C=O), 1460, 1380, 1360, 1250, 805 (—C=CH—). CD (EtOH): θ_{286} + 1419°, NMR: δ 0.78 (3H, *s*, Me), 0.83, 0.96 (9H each, *s*, 6 × Me), 1.10 (3H, *s*, Me), 3.50 (1H, C<u>H</u>OH), 5.21 (1H, *m*, —C=C<u>H</u>). MS *m/e* (rel. int.): 440 (M^+ 30), 425 (10), 407 (1.6), 311 (1.4), 272 (1.6), 257 (3), 235 (7.4), 218 (100), 203 (36), 189 (12), 177 (6.4), 175 (6.4), 161 (6.9), 149 (8.3), 147 (7.1), 137 (8.3), 135 (10), 133 (9.2), 121 (11.1), 119 (15.4). (Found: C, 81.78; H, 10.94 $C_{30}H_{48}O_2$ requires C, 81.81; H, 10.99%).

Castanopsone acetate. Castanopsone (50 mg) was reacted overnight with Ac_2O (0.5 ml) and dry C_5H_5N (0.5 ml) at room temp. The residue crystallised from MeCN as colourless needles, mp 184°, R_f 0.38 ($CHCl_3$–MeOH, 99.5: 0.5) NMR: δ 0.81 (3H, *s*, Me), 0.85 (9H, *s*, 3 × Me), 0.96 (3H, *s*, Me), 1.05 (6H, *s*, 2 × Me), 1.1 (3H, *s*, Me), 2.03 (3H, *s*, —OCOMe) 4.81 (1H, *q*, J_{aa} 10 Hz, J_{ae} 5 Hz, —CHOAc), 5.23 (1H, *t*, —C=C<u>H</u>—). MS *m/e*: 482 (M^+).

Castanopsone dinitrophenylhydrazone. Castanopsone (40 mg) in MeOH and 2,4 dinitrophenylhydrazine reagent (0.8 ml) were kept together for 15 min. Orange crystals, 35 mg, mp 245–50°, R_f 0.61 ($CHCl_3$–MeOH, 97:3). ν_{max}^{KBr} cm^{-1}: 3282, 1040 (OH), 2932, 1619 (C=N), 1590, 1336, 929, 840, 749 (aromatic).

$NaBH_4$ reduction of castanopsone. The substance (100 mg) and $NaBH_4$ (100 mg) in MeOH were stirred for 3 hr at room temp. and then worked up. The residue (105 mg) showed one major spot on TLC and crystallised from MeCN, mp 205°, R_f 0.33 ($CHCl_3$–MeOH, 97:3). ν_{max}^{KBr} cm^{-1}: 3380, 1037, 1021 (OH), 2980, 2870, 1461, 1379, 1351, 810 (—C=CH—). NMR: δ 0.8 (3H, *s*, Me) 0.85 (9H, *s*, 3 × Me), 0.9, 0.96, 1.01, 1.1 (12H, *s*, 4 × Me), 3.7 (2H, *m*, 2 × C<u>H</u>OH), 5.2 (1H, *m*, —C=C<u>H</u>—). MS *m/e*: 442 (M^+).

Hydrogenation of castanopsone. Castanopsone (60 mg) in EtOH and PtO_2 were shaken in H_2 atmosphere for 3 hr. The reaction mixture was filtered, evapd and crystallised as colourless needles from MeCN, mp 205°, 55 mg, R_f 0.33 ($CHCl_3$–MeOH, 97:3). It did not depress the mp of the $NaBH_4$-reduced product.

Oxidation of castanopsone. To castanopsone (50 mg) in $CHCl_3$ was added pyridinium chlorochromate (50 mg) and the reaction mixture was stirred for 4 hr at room temp. It was filtered through Si gel and the residue (35 mg), R_f 0.5 (C_6H_6), crystallised from hexane as colourless needles mp 198–99°. λ_{max}^{MeOH}: 259 nm; (MeOH/NaOH): 289 nm, ν_{max}^{KBr} cm^{-1}: 2940, 2854, 1720, 1700 (β diketo), 1455, 1375, 1258, 810. NMR: δ 0.91–1.2 (24H, 8 × Me), 3.36 (2H, *d*, quenched with D_2O, —CO—C<u>H</u>$_2$—CO—), 4.91 (1H, *m*, —C=C<u>H</u>—).

Wolff–Kishner reduction of castanopsone. Castanopsone (66 mg) was heated at 110° for 3 hr in diethylene glycol (6.6 ml) containing dry NH_2NH_2 (1 ml) and Na (176 mg). The mixture was further refluxed at 190° for 3 hr, cooled, diluted with H_2O and extracted with C_6H_6. The solvent layer yielded a residue showing a major spot, R_f 0.67 ($CHCl_3$–MeOH, 99:1), which was separated on a Si gel column. The C_6H_6 eluate yielded a product (34 mg) which crystallised from alcohol, mp 153–5° (lit. 152–3°). ν_{max}^{KBr} cm^{-1}: 2940, 2870, 1680 (αβΔ-ketone). λ_{max}^{MeOH}: 232 nm. NMR: δ 0.8–1.1 (24H, 8 × Me), 5.26 (1H, *m*, —C=C<u>H</u>—), 5.67, 6.36 (1H, each *d*, *J* = 10 Hz).

Dehydration of castanopsone. Substance (46 mg) in dry C_5H_5N (0.4 ml) and $MeSO_2Cl$ (0.4 ml) was kept at 0° for 24 hr. The soln was poured into H_2O and extracted with Et_2O to give the methane sulphonate. This material in C_6H_6 (4 ml) was stirred with basic Al_2O_3 (1 g) for 12 hr, filtered and washed with Et_2O. The filtrate was evapd and the residue (37 mg) showed two spots (R_f 0.8 major, 0.2 in C_6H_6) along with some original substance which were separated by PLC. The main component, 25 mg, crystallised from alcohol, mp 153–5°.

$NaBH_4$ reduction of castanopsone acetate. Castanopsone acetate (36 mg) in methanolic soln (3 ml) was stirred with $NaBH_4$ (30 mg) for 2 hr. The reaction mixture yielded a residue (36 mg) showing one major spot (R_f 0.5, $CHCl_3$–MeOH, 99:1) which was obtained by PLC and crystallised from MeCN, 18 mg, mp 159–60°. NMR: δ 0.8–1.12 (24H 8 × Me), 1.98 (3H, *s*, OCOMe), 3.59 (1H, *t*, $W_{1/2}$ = 7 cps, C<u>H</u>OH), 4.92 (1H, *q*, *J* = 6, 11 Hz, CHOAc), 5.14 (1H, *t*, —C=C<u>H</u>—).

Substance I (ursolic acid). Mp 280–85°, R_f 0.46 ($CHCl_3$–MeOH, 97:3). ν_{max}^{KBr} cm^{-1}: 3400, 1045, (OH), 1700 (COOH), 830. Methyl ester, mp 192–4° MS *m/e*: 470 (M^+). Monoacetate, mp 290–91°, MS *m/e*: 498 (M^+).

Substance J (castanopsol). Colourless needles, mp 204–5°, $[\alpha]_D$ + 71.8° (*c*, 0.8, $CHCl_3$), MS *m/e*: 442(M^+), 218 (base peak), 223, 203, 189, 177, 175, 133. (Found: C, 81.23; H, 11.53 $C_{30}H_{50}O_2$ requires C, 81.44; H, 11.31%).

Acetylation of castanopsol. Castanopsol (54 mg) was kept in Ac_2O (0.5 ml) and C_5H_5N (0.5 ml) for 17 hr at room temp. The derivative crystallised as colourless needles, mp 188–9°, R_f 0.81 ($CHCl_3$–MeOH, 99:1). NMR: δ 0.8–1.1 (24H, 8 × Me), 1.98, 2.06 (3H, *s*, 2 × OCOMe), 4.8 (2H, 2 × CHOAc), 5.11 (1H, *m*, —C=C<u>H</u>—). MS *m/e*: 526 (M^+).

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REFERENCES

1. Arthur, H. R. and Ko, P. D. S. (1968) *Australian J. Chem.* **21**, 2583.
2. Arthur, H. R. and Ko, P. D. S. (1969) *Australian J. Chem.* **22**, 597.
3. Hui, W. H. and Li, M. M. (1976) *Phytochemistry* **15**, 427.
4. Dhar, M. L., Dhawan, B. N., Prasad, C. R., Rastogi, R. P., Singh, K. K. and Tandon, J. S. (1974) *Indian J. Exp. Biol.*, **12**, 512.
5. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
6. Barton, D. H. R., Lier, L. F. and McGhie J. F. (1968) *J. Chem. Soc.* (*C*) 1031.
7. Narayanan, C. R., Pachapukkar, R. V., Sawant, B. M. and Wadia, M. S. (1969) *Indian J. Chem.* **7**, 187.
8. Allen, J., Boa, R. B., McGhie, J. F. and Barton, D. H. R. (1973) *J. Chem. Soc. Perkin Trans. 1*, 2402.
9. Hui, W. H. and Li, M. M. (1975) *Phytochemistry* **14**, 785.
10. Huneck, S. (1965) *Chem. Ber.* **98**, 2291.
11. Ganguli, A. K., Govindachari, T. R. and Mohammed, P. A. (1966) *Tetrahedron* **22**, 1513.