CHEMICAL CONSTITUENTS OF LEAVES AND ROOTS OF *GYMNOSPORIA MONTANA*

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Gymnosporia montana (Benth.) is used in indigenous medicine as a cure for ulcers, gastrointestinal troubles, dysentery, snake bite, etc. (1, 2). Joshi *et al.* (3) reported the isolation of tingenone, 3-O-acetyloleanolic acid, hexacosane, betulin, β-amyrin, hexacosanol and β-sitosterol from the stem bark, root bark, and leaves of *G. montana*. Anjaneyulu *et al.* (4) isolated β-amyrin from the stem of this plant; from the roots of the same plant, β-amyrin and dukidol were isolated by Satyanarayana and Mishra (5). Now, we report the isolation of β-amyrin and *n*-triacontanol from the leaves of *G. montana* (Benth.) and (−)epigallocatechin from the roots. Previously, (−)epigallocatechin was isolated from a very limited number of plants (6–13). Since this is the first report of the isolation of (−) epigallocatechin from the *Gymnosporia* species, it may be chemotaxonomically significant. Further, the isolation of *n*-triacontanol in high yields (0.107%) is significant since it was reported to possess a plant growth promoting property (14). β-Amyrin was identified by the preparation of its acetate and the comparison (mp, mmp and super-imposable ir) of both the compounds with authentic samples of β-amyrin and its acetate, available in our laboratories. *n*-Triacontanol was first isolated by crystallization. Since it is likely that *n*-triacontanol generally co-occurs with its homologous alkanols, the product was further purified by chromatography over silica gel. The acetate of *n*-triacontanol was prepared. The mass spectrum of *n*-triacontanol was recorded and found to be similar to the mass spectrum reported by Rice *et al.* (14). Fragment ions M-H_2O, M-CH_2OH and the consecutive loss of methylene units from the parent molecular ion were observed (14) in the ms of *n*-triacontanol. *n*-Triacontanol and its acetate were found to be identical (mp, mmp, ir) with authentic samples. The structure of (−)epigallocatechin was established by the preparation of the methyl ether, the methyl ether acetate, and the peracetate and comparison of their analytical and spectral data with those reported in literature (15, 16). However, an authentic sample of (−)epigallocatechin could not be procured. The mass spectrum of (−)epigallocatechin pentamethyl ether was recorded; its mass spectral fragmentation (376 (43)$M^{+\cdot}$, 358 (7), 210 (92), 181 (76), 167 (100), 152 (15)) is in agreement with those reported for catechin (6).

EXPERIMENTAL

Plant material.—*Gymnosporia montana* (Benth.) is a much-branched spinescent shrub occurring throughout the drier parts of India. The plant material was collected locally in September, 1976.

Isolation and identification of β-amyrin and *n*-triacontanol from the leaves of *Gymnosporia montana* (Benth).—The shade-dried leaves (1.5 kg) were extracted with petroleum ether (60–80°) (5 liters) in a Soxhlet extractor, and the extract was concentrated *in vacuo*. The resulting gummy mass (6.0 g) was treated with boiling methanol (2 liters), and then the extract was chilled. The waxy material that separated out was filtered and crystallized from a benzene-ethyl acetate (8:2) mixture as colorless needles of *n*-triacontanol. The product was further purified by chromatography over silica gel (70 g, Acme's 200 mesh). The details of the eluent fractions collected are given in table 1. The pure *n*-triacontanol obtained from the fractions

TABLE 1. The eluent fractions collected from column chromatography of *n*-triacontanol.

Sl. No.	Eluent System	Fraction numbers	Volume of each fraction (ml)	Remarks
1.	Petroleum ether (60–80°)	1–5	50	Yielded negligible amount of silky oily matter.
2.	Petroleum ether (60–80°)-benzene (8:2)	6–10	50	"
3.	Petroleum ether (60–80°)-benzene (7:3)	11–15	50	"
4.	Petroleum ether (60–80°)-benzene (1:1)	16 & 17	50	Yielded negligible amount of n-triacontanol along with silky oily matter.
5.	Petroleum ether (60–80°)-benzene (1:1)	18–20	50	Yielded pure n-triacontanol (0.6 g)
6.	Benzene	21–25	50	Yielded pure n-triacontanol (1.1 g)
7.	Benzene-chloroform (8:2)	26–30	50	Yielded negligible amount of silky oily matter.
8.	Benzene-chloroform (7:3)	31–35	50	"
9.	Benzene-chloroform (1:1)	36–40	50	"
10.	Chloroform	41–45	50	"

18 to 25 was again recrystallized from a benzene-ethyl acetate (8:2) mixture. It crystallized as colorless needles (1.6 g; 0.107%), mp 86° [lit. (17) mp 86°], M^+ 438(3). In the ms of *n*-triacontanol the following prominent ions were observed: *m/e* 420(33) ($M-H_2O$); 407(3) ($M-CH_2OH$); 393(5) ($M-CH_2OH-CH_2$) and ions due to consecutive loss of fourteen mass units (loss of CH_2 fragments) from *m/e* 393 ion. Anal. Calcd. for $C_{30}H_{62}O$: C, 82.19; H, 14.15. Found: C, 82.02; H, 14.23.

n-Triacontanol was acetylated with acetic anhydride and pyridine at room temperature. The acetate derivative crystallized from ethanol as colorless needles, mp 68.5° [lit. (17) 68.5°]. *n*-Triacontanol and its acetate were found to be identical (mp, mmp, tlc and superimposable ir) with respective authentic samples. Anal. Calcd. for $C_{32}H_{64}O_2$: C, 80.00; H, 13.34. Found: C, 79.92; H, 13.39.

The methanol solution (2 liters) was concentrated to 250 ml under reduced pressure and left for two days. The brown solid that separated out was filtered and crystallized from a chloroform-methanol (7:3) mixture as colorless needles of β-amyrin (3.0 g, 0.2%) mp 195° [lit. (18) mp 195°]. Anal. Calcd. for $C_{30}H_{50}O$: C, 84.50; H, 11.74. Found: C, 84.39; H, 11.79. It was acetylated with acetic anhydride and pyridine at room temperature. The acetate derivative crystallized from alcohol as colorless needles, mp 238°, [lit. (18) 238°]. The compounds were identical (mp, mmp, tlc, ir) with the corresponding authentic samples of β-amyrin and its acetate derivative. Anal. Calcd. for $C_{32}H_{52}O_2$: C, 82.12; H, 11.11. Found: C, 82.00; H, 11.16.

ISOLATION AND IDENTIFICATION OF (−)EPIGALLOCATECHIN FROM THE ROOTS OF *Gymnosporia montana* (Benth.).—The method of Seshadri *et al.* (19) was adopted for the isolation of (−)epigallocatechin. The shade-dried roots (0.5 kg) were defatted with petroleum ether (60–80°) and then extracted with acetone (3.0 liters) in a Soxhlet extractor. The acetone extract was concentrated *in vacuo*, and the resulting mass (2.0 g) was dissolved in water (250 ml) and filtered. The aqueous solution was extracted with ether (2 x 200 ml) and than with ethyl acetate (2 x 200 ml). The combined ethyl acetate extract was dried and concentrated under reduced pressure to a small volume (10.0 ml). To this extract 4 ml of petroleum ether (60–80°) was added with stirring; a brown gummy mass separated out. When the clear solution was decanted into another flask and 4 ml of petroleum ether (60–80°) was added, a solid again separated out. This process of fractional precipitation was repeated eight more times. The colorless solid fractions obtained in the last three operations were found to be identical in nature when subjected to paper chromatography: Whatman No. I chromatographic paper, solvent

TABLE 2. The eluent fractions collected from column chromatography of pentamethyl ether of (−) epitallocatechin.

Sl. No.	Eluent System	Fraction numbers	Volume of each fraction (ml)	Remarks
1.	Benzene	1–5	50	Yielded negligible amount of mixture of compounds.
2.	Benzene-ethyl acetate (9:1)	6–10	50	"
3.	Benzene-ethyl acetate (7:3)	11–15	50	Yielded pure pentamethyl ether of (−) epigallocatechin.
4.	Benzene-ethyl acetate (1:1)	16–20	50	Yielded negligible amount of mixture of compounds.
5.	Ethyl acetate	21–25	50	"

system butanol-acetic acid-water (4:1:5 upper layer, spray reagent alcoholic ferric chloride, R_f 0.52). Therefore, they were mixed and dissolved in ethyl acetate (8.0 ml). The process of fractional precipitation with dry petroleum ether (60–80°) was repeated until almost colorless (−)epigallocatechin was obtained (1.5 g; 0.3%), mp 216–18° (d) [lit. (15, 16) mp 218–19°]; uv λ max (MeOH), nm (log ε): 224, 271, 224 (4.36), 271 (3.184) [lit. (15, 16)]. Anal. Calcd. for $C_{15}H_{14}O_7$: C, 58.83; H, 4.58. Found: C, 58.72; H, 4.64.

PENTAMETHYL ETHER OF (−)EPIGALLOCATECHIN.—(−)Epigallocatechin was methylated with dimethylsulfate and potassium carbonate in dry acetone. The methyl ether was chromatographed over silica gel (Acme's 200 mesh). The details of the eluent fractions are given in table 2. Pure pentamethyl ether obtained from the fractions 11 to 15 was recrystallized from benzene-ethyl acetate (8:2) mixture as colorless crystals, mp 157–59° [lit. (15, 16) mp 158–60°]; uv λ max (MeOH) nm 272 (log ε) (3.60); ir ν max (KBr) 3450 cm^{-1} (hydroxyl); $[\alpha]^{25}D$ −16.5° acetone [lit. (15, 16) $[\alpha]^{25}D$ −15.2° acetone]; ms 376 (43) M^+ 358 (7), 210 (92), 181 (76), 167 (100), 152 (15). Anal. Calcd. for $C_{20}H_{24}O_7$: C, 63.83; H, 6.38; OCH_3, 41.22. Found: C, 63.74; H, 6.39, OCH_3, 41.02.

PENTAMETHYL ETHER MONOACETATE OF (−)EPIGALLOCATECHIN.—The pentamethyl ether of (−)epigallocatechin was acetylated with acetic anhydride and pyridine at room temperature. The acetate derivative crystallized from benzene-ethyl acetate (8:2) mixture as colorless needles, mp 179–80° [lit. (15, 16) mp 181–83°]; uv λ max (MeOH) nm (log ε) 272 (3.30); ir ν max (KBr) 1750 cm^{-1} (acetate carbonyl). Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.16; H, 6.22. Found: C, 63.05; H, 6.28.

HEXAACETATE OF (−)EPIGALLOCATECHIN.—(−)Epigallocatechin was acetylated with acetic anhydride and pyridine at room temperature. The acetate derivative crystallized from a benzene-ethyl acetate (8:2) mixture as colorless needles, mp 190–92° [lit. (15, 16) mp 191–93°]; uv λ max (MeOH) nm (log ε) 272 (3.54); ir ν max 1750 cm^{-1} (acetate carbonyl); $[\alpha]^{25}D$ −14.00° acetone [lit. (15, 16) $[\alpha]^{25}D$ −14.00° acetone]. Anal. Calcd. for $C_{27}H_{26}O_{13}$: C, 58.06; H, 4.66. Found: C, 57.95; H, 4.71.

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