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STUDIES ON TINOSPORA CORDIFOLIA MIERS (BENG: GULANCHA)

Part IV.—Isolation of Heptacosanol, β-sitosterol and Three Other Compounds Tinosporidine, Cordifol and Cordifolone

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Petroleum-ether extraction of the dry stems of *Tinospora cordifolia* yielded heptacosanol and a compound, m.p. 233–235°C, which has been tentatively named cordifol. From the alcoholic extract of the fresh stems, β-sitosterol and a compound $C_{32}H_{64}O_3$, m.p. 94–97°C, have been isolated. The name *tinosporidine* has been assigned to this compound. Dry leaves of *Tinospora cordifolia*, on extraction with petroleum ether, yielded heptacosanol and a carbonyl compound $C_{33}H_{64}O$, m.p. 62–62.5°C, tentatively named cordifolone.

In the previous communication[1] a brief description on the isolation of tinosporidine, cordifol, heptacosanol and β-sitosterol was reported. The present paper deals with the detailed work on these compounds as well as another compound cordifolone.

Fresh stems, of *Tinospora cordifolia* were extracted with 95% ethanol. Solvent was partially distilled and the semiconcentrated solution, on standing, yielded a brown material. This was subjected to alumina chromatography and the chloroform-eluted fraction gave tinosporidine $C_{32}H_{64}O_3$, m.p. 94–97°C. It is soluble in chloroform, acetone, ethanol, methanol and insoluble in ether and petroleum-ether. After separating tinosporidine, from the remaining mother liquor a compound m.p. 135–137°C, was separated and identified to be β-sitosterol from the studies of Liebermann-Burchard test, identical IR absorption spectra and mixed melting point with a sample of β-sitosterol from *Cephalandra indica.*[2]

Petroleum ether extract of the dry stems of *Tinospora cordifolia* yielded a dark semi solid mass which on purification through alumina chromatography and crystallisation, yielded a compound m.p. 233–235°C, tentatively named cordifol. Though the melting point of cordifol is close to that of tinosporide (m.p. 236–238°C, previously reported)[3] the mixed melting point (225–228°C) of these two compounds show that the former is different from tinosporide. Solubilities of these two compounds are also different. In a separate batch, dry stems of *Tinospora cordifolia,* on extraction with petroleum ether and subsequent chromatography of the concentrated extract over alumina and crystallisation of the chromatographic fractions, yielded heptacosanol $C_{27}H_{55}OH$.[4] Powdered dry leaves of *Tinospora cordifolia,* on extraction with petroleum ether, also yielded heptacosanol and a carbonyl compound $C_{33}H_{64}O$, m.p. 62–62.5°C tentatively named cordifolone.

Experimental

Isolation of Tinosporidine.—Fresh stems of *Tinospora cordifolia* (26 kg) were crushed into small pieces and extracted exhaustively with rectified spirit. The solvent was distilled largely to a semi concentrated state. On standing, a flocculent precipitate separated out from the solution. This was filtered (7.97 g) and subjected to a chromatography over neutral alumina (10 times) with benzene as the starting eluent. The chloroform-eluted fraction (light yellow solid; 0.38 g) was purified by repeated crystallisations from acetone when a constant melting point (94–97°C) was attained. A sample was analysed (Found: C, 77.67; H, 12.80; O, 9.59%; mol.wt. 482. Tinosporidine $C_{32}H_{64}O_3$ requires C, 77.48; H, 13.01; O, 9.68%; mol.wt. 496). It is soluble in benzene, chloroform, acetone, ethanol, methanol and insoluble in ether and petroleum ether. It has characteristic IR absorption spectrum in potassium bromide pellet: ν_{max} 3450(OH), 2960, 2880, 1465, 1387, 729, 718 cm^{-1} (Fig. 1).

Isolation of β-sitosterol.—After the separation of tinosporidine (mentioned above) the mother liquor was acidified and extracted with petroleum-ether (60–80°C) which yielded a viscous green oily material (12.41 g). This was subjected to alumina

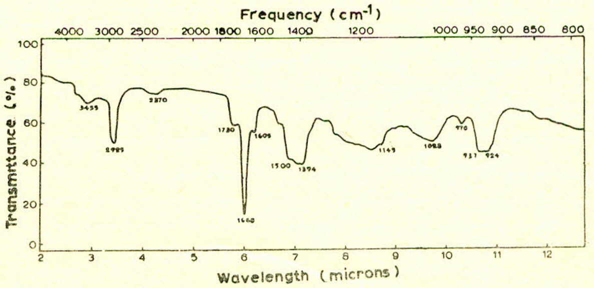

Fig. 1.—IR absorption spectrum of tinosporidine.

chromatography (20 times). The materials in fraction Nos. 8–11 (eluted with petroleum-ether–benzene mixture) were of similar nature and these were combined and purified by repeated crystallisations from ethanol (m.p. 135–137°C). This substance responded to Liebermann-Burchard test for sterols. Mixed melting point of this with an authentic sample of β-sitosterol from *Cephalendra indica*,[2] was undepressed and IR absorption spectra were also identical.

Isolation of Cordifol.—Dry powdered stem of *Tinospora cordifolia* (8 kg) was extracted with petroleum ether (60–80°C). On distillation of solvent, the dark semisolid mass (95 g) was chromatographed over neutral alumina (8 times) with petroleum-ether as the starting eluent. The benzene-eluted fraction (1.5 g; m.p. 229–230°C) yielded a crystalline substance which was purified by repeated crystallisations from petroleum ether–chloroform mixture to a constant melting compound (233–235°C, 0.018%). It has characteristic IR spectrum (chloroform): ν_{max} 3455(OH), 1730(C = O), 1668 (C = O), 1605 (C = C), 1500(C = C), 1394, 1028, 970, 937 and 924 cm^{-1} (Fig. 2).[5] It is soluble in chloroform but sparingly soluble in petroleum ether. The melting point of this compound is very close to that of tinosporide (m.p. 236–238°C, previously reported).[3] However, the mixed melting point of the two compounds was depressed (m.p. 225–228°C). Solubility difference of the compounds also indicate that they are different compounds. The name cordifol has been assigned to this compound.

Isolation of Heptacosanol.—In a separate batch, dry powdered stem of *Tinospora cordifolia* (5.21 kg) was extracted exhaustively with petroleum-ether (60–80°C). Solvent was distilled off when a yellowish-brown waxy material (54.18 g) was obtained. This was chromatographed over neutral aluminia (15 times) with petroleum–ether (40–60°C) as a starting eluent. The fractions 10–17 (eluted by petroleum ether–benzene mixture and benzene) were combined (12.37 g) and rechromatographed over neutral alumina. The fractions, thus eluted, were repeatedly crystallised from petroleum ether–benzene mixture to a constant melting compound (m.p. 79–80°C; 0.18%). A sample was analysed (Found: C, 82.33; H, 13.71; O, 4.29; Act. H^+ 0.37, C—CH_3, 2.46%; mol.wt. 391. Heptacosanol $C_{27}H_{56}O$ requires, C, 81.89; H, 14.25; O, 4.04; C—CH_3 (one), 3.79; Act. H^+, 0.25%; mol.wt. 396). It has IR spectrum (chloroform): ν_{max} 3550 (OH), 2935, 2875, 1465, 1350, 1123 cm^{-1}. Heptacosanol was further confirmed by its acetylation with acetic anhydride in pyridine.

Heptacosanol Acetate.—Heptacosanol (0.2 g) was dissolved in dry pyridine (5 ml) on heating and distilled acetic anhydride (0.5 ml) was slowly added to it. The mixture was heated at a temperature of 100°C for 6 hr after which solvent was completely removed under vacuum when a slightly coloured solid substance was obtained. This was purified by 6 crystallisations from petroleum ether–acetone mixture (m.p. 66–67°C) and analysed (Found: C, 80.06; H, 13.27; acetyl value, 4.91; C—CH_3, 2.83%; mol.wt. 453. Heptacosanol acetate $C_{29}H_{58}O_2$ requires C, 79.52; H, 13.35; acetyl value, 9.81; C—CH_3, 3.42%; mol.wt. 438). It has acetate peaks at 1740 and 1245 cm^{-1} in the IR spectrum.

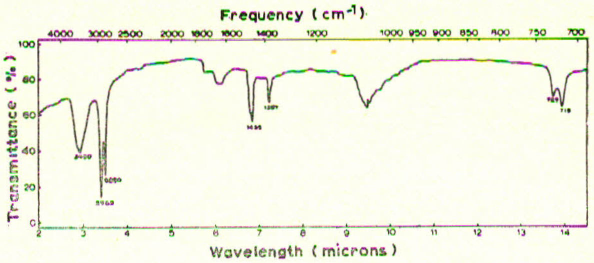

Fig. 2.—IR absorption spectrum of cordifol.

Heptacosanol and β-sitosterol from the Leaves.—Powdered leaves of *Tinospora cordifolia* (5.13 kg) were extracted with petroleum ether and on removal of solvent the dark semisolid mass (180 g; 2.83%) was chromatographed over neutral alumina. Fractions 2–5 (eluted with petroleum ether) on repeated crystallisations from ether, yielded a compound m.p. 79–80°C and was identified to be heptacosanol by comparison of its mixed melting point with that of heptacosanol isolated previously from *Tinospora cordifolia* stems (see above). From the fractions 8–9 of the same chromatography another compound m.p. 134.5–135°C was obtained. It responded to the tests for sterols and was identified to be β-sitosterol by comparison with an authentic sample of *Terminalia arjuna*[6] (mixed m.p. was undepressed).

Isolation of Cordifolone.—In a separate batch the dry powdered leaves (0.38 kg) of the plant were extracted exhaustively with petroleum ether (60–80°C). On distillation of solvent, a semisolid mass (7.79 g) was obtained and chromatographed over neutral alumina. The petroleum ether–eluted fractions were combined (1.79 g; m.p. 50–60°C) and purified by repeated crystallisations from chloroform-acetone mixture when it melted at 62–62.5°C. A sample was analysed (Found: C, 83.47; H, 13.35; O, 3.85; C—CH_3, 7.4; Act. H^+, 0.125%; mol.wt. 537. Cordifolone $C_{33}H_{64}O$, requires C, 83.26; H, 13.35; O, 3.36; C—CH_3, 3.15; Act. H^+, 0.21%; mol.wt. 476). It is soluble in petroleum ether, acetone and chloroform. It gives IR absorption peaks at 1720 cm^{-1} (C = O) and no peak in the hydroxyl region.

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