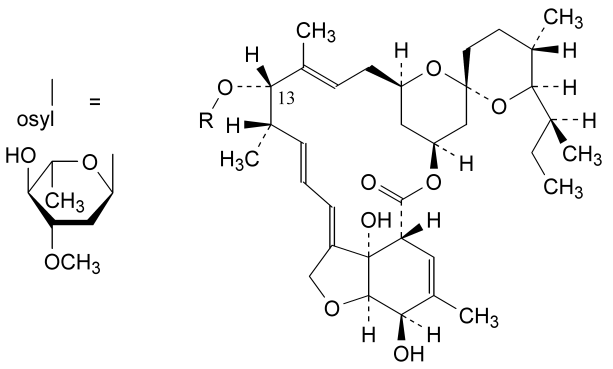
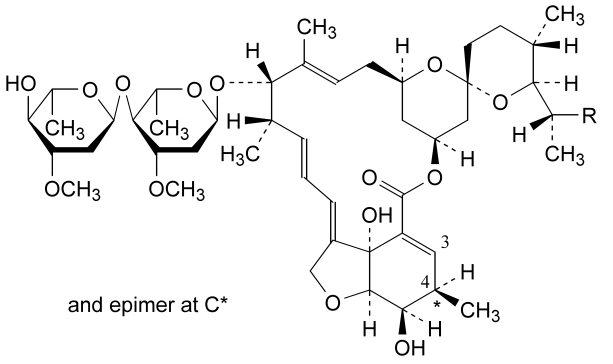


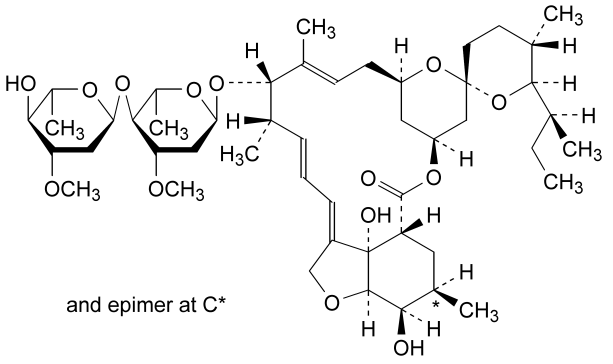
G. R = H: (6*R*,13*S*,25*R*)-5-*O*-demethyl-28-deoxy-6,28-epoxy-13-hydroxy-25-[(1*S*)-1-methylpropyl]milbemycin B (H_2B_{1a} aglycone),

H. R = osyl: 4′-*O*-de(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-5-*O*-demethyl-22,23-dihydroavermectin A_{1a},



I. R = C_2H_5: 2,3-didehydro-5-*O*-demethyl-3,4,22,23-tetrahydroavermectin A_{1a} ($\Delta^{2,3}$ H_2B_{1a}),

J. R = CH_3: 2,3-didehydro-5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-3,4,22,23-tetrahydroavermectin A_{1a} ($\Delta^{2,3}$ H_2B_{1b}),



K. (4*R*) and (4*S*)-5-*O*-demethyl-3,4,22,23-tetrahydroavermectin A_{1a} (H_4B_{1a} isomers).

01/2008:2148
corrected 6.0

IVY LEAF

Hederae folium

DEFINITION

Whole or cut, dried leaves of *Hedera helix* L., collected in spring.

Content: minimum 3.0 per cent of hederacoside C ($C_{59}H_{96}O_{26}$; M_r 1221) (dried drug).

IDENTIFICATION

A. Whole leaves are coriaceous, 4-10 cm in length and width, cordate at the base. The lamina is palmately 3-5 lobed, the lobes more or less triangular with entire margins. The upper surface is dark green with a paler, radiate venation, the lower surface more greyish-green and the venation is distinctly raised. The petioles are long, cylindrical, about 2 mm in diameter and grooved longitudinally. Scattered white hairs occur on the petioles and on the surfaces of younger leaves, the older leaves are glabrous. Occasional entire, ovate-rhombic to lanceolate leaves 3-8 cm long from the flowering stems may be present.

B. Reduce to a powder (355) (*2.9.12*). The powder is green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the lamina in surface view showing the cells of both the upper and lower epidermises with thickened, sinuous to wavy anticlinal walls and a thick cuticle; numerous stomata occur in the lower epidermis only, they are mostly anomocytic but occasionally anisocytic (*2.8.3*) and some of the surrounding cells may show faint cuticular striations; scattered stellate covering trichomes may be present, composed of 4-8 unicellular branches joined at the base; fragments in sectional view show 1-3 (usually 2) layers of palisade cells and a very porous spongy mesophyll containing occasional large mucilage cells; cluster crystals of calcium oxalate, about 40 µm in diameter, occur throughout the mesophyll; groups of lignified fibro-vascular tissue from the veins.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Extract 0.50 g of the powdered drug (355) (*2.9.12*) under a reflux condenser in a water bath at 60 °C with 5 ml of *methanol R* for 30 min. Cool and filter.

Reference solution. Dissolve 1.0 mg of *hederacoside C R* and 1.0 mg of *α-hederin R* in 1.0 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, methanol R, acetone R, ethyl acetate R* (4:20:20:30 *V/V/V/V*).

Application: 20 µl, as bands of 15 mm.

Development: over a path of 12 cm.

Drying: at 100-105 °C.

Detection: spray with *alcoholic solution of sulphuric acid R*. Heat at 110 °C for 10 min. Examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A green zone
———	———
α-Hederin: a purple zone	A very faint purple zone (α-hederin)
	A broad yellow zone
	2-3 purple or green zones
———	———
Hederacoside C: a purple zone	A purple zone (hederacoside C)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 10 per cent of discoloured matter, maximum 10 per cent of stems, and maximum 2 per cent other foreign matter.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 10.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 1.00 g of the powdered drug (355) (*2.9.12*) in a 250 ml round bottomed flask add 50 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R*. Heat under a reflux condenser in a water-bath at 80 °C for 1 h. Cool and filter through cotton into a 100 ml volumetric flask. The cotton together with the residue is again extracted with 30 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* under reflux for 30 min. Filter and combine the filtrates. Rinse the round bottomed flask and the cotton with a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* and use this mixture of solvents to dilute the contents of the volumetric flask to exactly 100.0 ml. Filter through a suitable membrane before use.

Reference solution. Dissolve an amount of *ivy leaf standardised tincture CRS* corresponding to 3.0 mg of hederacoside C in *methanol R* and dilute to 5.0 ml with the same solvent.

Column:

– *size*: *l* = 0.125 m, Ø = 4 mm;
– *stationary phase*: suitable *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: mix 140 volumes of *acetonitrile R* and 880 volumes of *water R* and adjust to pH 2.0 with *phosphoric acid R*;
– *mobile phase B*: *phosphoric acid R, acetonitrile R* (2:998 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 6	100 → 94	0 → 6
6 - 40	94 → 60	6 → 40
40 - 41	60 → 0	40 → 100
41 - 55	0	100
55 - 56	0 → 94	100 → 6
56 - 70	94	6

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 205 nm.

Injection: 20 µl.

System suitability: reference solution:

– *retention time*: hederacoside C = about 20 min.

If necessary, adjust the time intervals of the gradient.

Calculate the percentage content of hederacoside C with reference to the dried drug using the following expression:

$$\frac{F_1 \times m_2 \times 400}{F_2 \times m_1 \times (100 - d)}$$

F_1 = area of the peak due to hederacoside C in the chromatogram obtained with the test solution;

F_2 = area of the peak due to hederacoside C in the chromatogram obtained with the reference solution;

d = loss on drying, in per cent;

m_1 = mass of drug, in grams;

m_2 = mass of hederacoside C in the reference solution, in grams.