PLANT ANTICANCER AGENTS X. ISOLATION OF CAMPTOTHECIN AND 9-METHOXYCAMPTOTHECIN FROM *ERVATAMIA HEYNEANA*[1]

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ABSTRACT.—The anticancer activity of the roots of *Ervatamia heyneana* (Apocynaceae) was found to be due principally to the known alkaloid, camptothecin (**1**). The closely related, but less active, 9-methoxycamptothecin (**2**) was also obtained. This is the first reported isolation of these biogenetically interesting alkaloids from the indole alkaloid rich Apocynaceae.

Aqueous alcoholic extracts of the wood and stem bark of *Ervatamia heyneana* (Wall.) T. Cooke (Apocynaceae) exhibited activity[2] in the P-388 lymphocytic leukemia system *in vivo* and Eagles carcinoma of the nasopharynx (KB) system in cell culture. Fractionation with concomitant bioassay afforded several active alkaloids (3), but the principal source of activity was traced to a neutral component identified as camptothecin (**1**). Chromatography of the mother liquor, after removal of **1**, afforded 9-methoxycamptothecin (**2**). The compounds were identified by comparison with authentic samples.

EXPERIMENTAL[3]

PLANT MATERIAL.—The plant material[4] was collected in India in December 1976.

EXTRACTION AND INITIAL FRACTIONATION.—A sample (35 kg) of the wood and stem bark of *E. heyneana* was extracted successively with light petroleum and methanol; after evaporation *in vacuo*, the residues weighed 180 g and 960 g, respectively. The methanol extract was partitioned between water (3 liters) and ethyl acetate (3 liters); after drying (Na_2SO_4), filtering, and evaporating *in vacuo* the ethyl acetate residue weighed 115 g. Separation of the alkaloid fraction from this material was accomplished by extraction with 5% tartaric acid (3×500 ml); and the neutral fraction, after evaporation, weighed 107.2 g.

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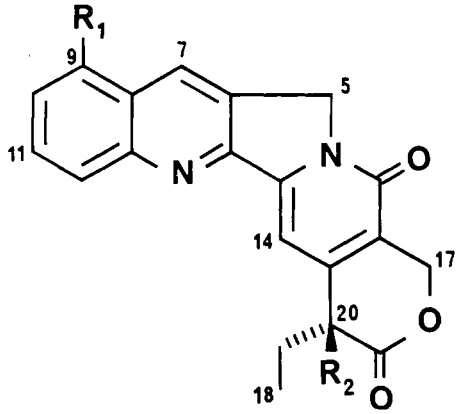
[1]For the previous paper in this series see Ref. 1.

[2]Extracts, fractions and compounds were tested under the auspices of the Drug Research and Development Program of the National Cancer Institute (2). An isolate is considered active if it shows a T/C value of greater than 130% in the P-388 lymphocytic leukemia system in mice or an ED_{50} of 4 μg/ml or less in the KB or P-388 test systems in cell culture.

[3]Melting points were determined using a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrophotometer. The ir spectra were obtained with a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Pmr spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions with a Varian model T-60A instrument, operating at 60 MHz with a Nicolet, model TT-7, Fourier Transform attachment. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ (ppm) units. Low resolution mass spectra were obtained with a Hitachi Perkin Elmer, model RMU-6D, single-focusing spectrometer operating at 70 ev.

[4]The plant material was supplied through the auspices of the Drug Research and Development Program, National Cancer Institute, by the Economic Botany Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Service, U.S.D.A., Beltsville, MD. A herbarium specimen documenting this collection is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

SEPARATION OF THE NEUTRAL FRACTION.—The neutral fraction (107 g) was chromatographed on a column of silica gel PF 254[5] (2 kg) packed in chloroform. Elution with chloroform-methanol (98:2) and concentration yielded a green residue (1.1 g) which, when rechromatographed on Florisil[6] (20 g) packed in chloroform, yielded crude camptothecin. Crystallization from chloroform afforded pale yellow crystals (45 mg, 0.00013%) of **1** having the following physical properties: mp 270–271° d, $[\alpha]^{25}D+42.8°$ (c. 0.28, $CHCl_3$-MeOH; 4:1) [Lit. (4) 268–270° d, $[\alpha]^{25}D+42°$ ($CHCl_3$-MeOH; 4:1)]; ir, νmax (KBr) 3420, 3250, 2940, 2915, 1745, 1655, 1605, 1585 and 1157 cm^{-1}; uv, λmax (MeOH) 368 (log ε 4.34), 358 (4.34), 289 (3.81), 253 (4.51), and 218 nm (4.63); pmr, (d_6-DMSO) δ 0.89 (3H, t, $J=6.9$ Hz, C-18 H), 1.88 (2H, q, $J=6.9$ Hz, C-19 H), 5.28 (2H, s, C-5 H), 5.42 (2H, s, C-17 H), 6.49 (1H, s, C-20 OH), 7.36 (1H, s, C-14 H), 7.68–8.31 (4H, m, C-9–12 H) and 8.66 (1H, s, C-7 H); ms, *m/e* M^- 348 (100%), 319 (24), 304 (20), 291 (13), 275 (14), 261 (3), 248 (21), 219 (19), 205 (8), 191 (9), 178 (5) and 140 (4). The compound was identical with an authentic natural sample kindly supplied by Drs. Wall and Wani.



	R_1	R_2
1	H	OH
2	OCH_3	OH
3	H	$OCOCH_3$

ACETYLATION OF CAMPTOTHECIN (**1**).—Camptothecin (**1**, 10 mg) was treated with pyridine (2 ml) and acetic anhydride (0.1 ml) at room temperature for 3 days. Work-up in the usual way followed by crystallization from hexane yielded acetyl camptothecin (**3**) as pale yellow needles, mp 289–290° d, [Lit. (4) 288–290° d]; ir, νmax (KBr) 3480, 1755, 1675, 1665, 1625 and 1235 cm^{-1}; uv, λmax (MeOH) 368 (log ε 4.07), 356 (4.07), 288 (3.52), 252 (4.26) and 218 nm (4.42); pmr, ($CDCl_3$) δ 0.97 (3H, t, $J=7.3$ Hz, C-18 H), 2.23 (2H, m, C-19 H), 2.31 (3H, s, $-OCOCH_3$), 5.28 (2H, s, C-5 H), 5.35 (1H, d, $J=16.6$ Hz, C-17 H), 5.73 (1H, d, $J=16.6$ Hz, C-17H), 7.25 (1H, s, C-14 H), 7.67–8.38 (5H, m, C-7, 9–12 H); ms *m/e* M^+ 390 (65%), 330 (96), 315 (50), 302 (100), 287 (48), 275 (20), 246 (15), 219 (15), 205 (12), and 191 (10).

ISOLATION AND CHARACTERIZATION OF 9-METHOXYCAMPTOTHECIN (**2**).—The mother liquor, after crystallization of camptothecin, was chromatographed on a plate of silica gel. A uv florescent band was separated and, on crystallization from chloroform-hexane (1:2), afforded yellow crystals of 9-methoxycampthothecin (**2**) (14 mg, 0.00004%) having the following physical properties: mp 258–259° $[\alpha]^{25}D-76.1°$ (c. 0.5, pyridine) [Lit. (7) mp 258–259°, $[\alpha]^{25}D-77.5°$]; ir, νmax (KBr) 3420, 2940, 1761, 1668, 1622, 1603, 1450, 1372, 1270, 1237, 1195, 1160, 1112, 1090, 1050 and 813 cm^{-1}; uv, λmax (MeOH) 371 sh (log ε 4.28), 356 (4.30), 320 (4.05), 305 (3.85), 262 (4.38) and 218 nm (4.52); pmr, ($CDCl_3$) δ 1.03 (3H, t, $J=7.4$ Hz, C-18 H), 1.91 (2H, q, $J=7.4$ Hz, C-19 H), 4.05 (3H, s, C-9 OCH_3), 5.25 (2H, s, C-5 H), 5.25 (1H, d, $J=16.4$ Hz, C-17 H), 5.76 (1H, d, $J=16.4$ Hz, C-17 H), 6.93 (1H, dd, $J=6.2$, 2.5 Hz, C-10 H), 7.64–7.77 (2H, m, C-11 and C-12 H), 7.74 (1H, bd, C-20 OH) and 8.78 (1H, s, C-7 H); ms, *m/e* M^+ 378 (100%), 350 (17), 349 (41), 334 (37), 321 (23), 319 (29), 305 (23), 278 (41), 249 (17), 206 (19), 205 (18), 139 (20), 103 (31) and 89 (10). The compound was identical with an authentic natural sample kindly supplied by Dr. Suffness.

BIOLOGICAL ACTIVITY.—Camptothecin (**1**, NSC-94600) showed T/C values of 181% and 175% at doses of 1.56 and 0.78 mg/kg, respectively, in the P-388 lymphocytic leukemia test system (2) and was cytotoxic in the KB and P-388 test systems in cell culture (ED_{50} 0.17 and 0.53 μg/ml respectively).

DISCUSSION

Camptothecin (**1**), an alkaloid derived biosynthetically (5, 6) from an indole

[5]E. Merck, Darmstadt, W. Germany.
[6]Fisher Scientific Co., Itasca, Il.

precursor, was first isolated from the Chinese tree *Camptotheca acuminata* Decne. (Nyssaceae) (4) and subsequently from *Nothapodytes foetida* (Wight) Sleumer (Icacinaceae) (7) and *Ophiorrhiza mungos* Linn. (Rubiaceae) (8). Besides having potent antileukemic and antitumor properties (4, 9), camptothecin also inhibits herpes (10) and other mammalian viruses (9).

9-Methoxycamptothecin (**2**, NSC-176323), formerly obtained from *N. foetida* (7), was not further evaluated biologically, but it has previously shown substantial activity in the B16 melanoma, L-1210 lymphoid leukemia, Lewis Lung carcinoma and P-388 lymphocytic leukemia test systems *in vivo* at doses in the range 0.5–2.0 mg kg (11).

This is the first reported isolation of this alkaloid type from the Apocynaceae.

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