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CHONEMORPHINE AND RAPANONE—ANTIPARASITIC AGENTS FROM PLANT SOURCES

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Our screening program in search of biologically active compounds from natural sources (1) was extended to look for novel, antiparasitic agents from plants. The initial goals of this extended program were compounds with antiamoebic and antitrichomonad properties. Plants were chosen for the program according to selection criteria based on traditional medicine, ethnotherapeutic reports, phylogenetic relationships, phytogeographic considerations, and random choice (1).

CH_2Cl_2, MeOH, and aqueous extracts of the selected plants, totalling about 2500 extracts in number, were screened for activity against the parasites *Entamoeba histolytica* and *Trichomonas vaginalis* in in vitro and in vivo models (2,3). Plant extracts that displayed activity were those of *Chonemorpha fragrans* (Moon) Alston (Syn. *C. macrophylla* G. Don) (Apocynaceae) and *Ardisia oxyphylla* Burm. (Myrsinaceae).[1] Selection of *C. fragrans* was based on two considerations, namely the use of the species in the Indian ayurvedic system of medicine for the treatment of parasitic infections (4), and phylogenetic relationship of the genus to *Holarrhena antidysenterica* Wall., the plant source for the antiamoebic, steroidal alkaloid, conessine (5). Selection of *A. oxyphylla* was based on its relationship to the allied species *Ardisia solanacea* Roxb., the roots of which are reported to be antidiarrheal, and secondly on its exclusive location in India on the Andaman Islands (6).

This paper describes the identification of chonemorphine as the antiamoebic principle of *C. fragrans*, and rapanone containing small amounts of embelin and the higher $C_{21}H_{34}O_4$ homologue as the antitrichomonad principle of *A. oxyphylla*. This sample of rapanone displayed in vitro activity against *E. histolytica* BY 80 (200 μg/ml) and *T. vaginalis* (200 μg/ml). Chonemorphine dihydrochloride displayed in vitro activity against *E. histolytica* BY 80 (25 μg/ml) and *T. vaginalis* (200 μg/ml), and in vivo activity against hepatic amoebiasis in golden hamsters (cured/treated= 14/14, 100 mg/kg×4 doses, p.o.) and intestinal amoebiasis in weanling Wistar rats (cured/treated=4/4, 200 mg/kg×4 doses, p.o.) (2).[2] This report, together with our associated publications, is the first report of the activity of these molecules (2,7). The compounds were identified by comparison of data (mp, ^{1}H nmr, ir, uv) with reported information on these compounds (8-10).

EXPERIMENTAL

PLANT MATERIALS.—The roots of *C. fragrans* and the roots of *A. oxyphylla* were collected from the forests of Meghalaya and South Andamans, respectively. Voucher specimens of both the species are preserved at the Herbarium of the Research Centre, Hoechst India Limited, Bombay 400 080, India.

ISOLATION OF CHONEMORPHINE DIHYDROCHLORIDE.—The roots (4.8 kg) of *C. fragrans* were extracted successively with MeOH (2×20 liters), 0.04% (w/v) NaOH in MeOH-H_2O (9:1), and 1% (w/v) HOAc in MeOH-H_2O (9:1)

[1]Out of 2541 extracts, 498 tested showed in vitro activity against *E. histolytica* (200 μg/ml, and 179 extracts out of 2381 tested showed in vitro activity against *T. vaginalis* (200 μg/ml) but were not considered for further investigation.

[2]For the standard drug metronidazole, the ED_{50} value against hepatic amoebiasis in golden hamsters is <40 mg/kg×4 doses, p.o., and against intestinal amoebiasis in weanling Wistar rats is 235 mg/kg×4 doses, p.o. (198-278) (p>95% limits).

(2×20 liters). The combined extracts were evaporated to dryness under vacuum. The residue was taken up in $CHCl_3$ (3 liters) and thoroughly extracted with 2N HCl (4×500 ml) until the last extract was free of alkaloids. The combined acid extracts were cooled to ca. 10° and basified to *p*H 9.0. The precipitate obtained was treated with MeOH. The MeOH-soluble portion (125 g) was found to display antiamoebic activity [ED_{50}, hepatic amoebiasis, golden hamsters (3): 200 mg/kg×4, p.o.] and was subjected to chromatography on an HP-20 column (length 130 cm, dia. 2.5 cm). Elution was carried out with H_2O (500 ml), subsequently, H_2O containing 10, 25, 50, 75% of MeOH (250 ml each), and finally, with MeOH (500 ml). Residues obtained from evaporation of the 10, 25, 50, and 75% MeOH-H_2O fractions displayed activity [ED_{50}, hepatic amoebiasis, golden hamsters (3): 200 mg/kg×4, p.o., for each fraction]. Crystallization of the combined active fractions (46 g) from MeOH-EtOAc (8:2) yielded chonemorphine dihydrochloride (32 g), mp>300°. Found: C, 60.72; H, 10.39; N, 6.31; Cl, 15.92%. Calculated for $C_{23}H_{42}N_2.2HCl.2H_2O$: C, 60.64; H, 10.64; N, 6.15; Cl, 15.92%.

Chonemorphine dihydrochloride (2 g) was dissolved in MeOH (30 ml) and the solution basified to *p*H 11.0 with aqueous NaOH. The resulting solution was evaporated to dryness in vacuo, and the residue was desalted on an HP-20 column. The MeOH eluate yielded a compound, which crystallized from EtOAc as colorless crystals (1.2 g), mp 144-145° [lit. (8) 144-146°], $[\alpha]^{25}D$: +24.3° [lit. (8)+25°]. The spectral data (ir, ^1H nmr) were found to be identical with those reported for chonemorphine (8,9).

ISOLATION OF RAPANONE.—The dried, ground, and defatted roots (1.5 kg) of *A. oxyphylla* were extracted with CH_2Cl_2. The active CH_2Cl_2 extract [50 g, EC_{100} (*E. histolytica* and *T. vaginalis*) (3): 200 μg/ml] was stirred with petroleum ether (bp 60-80°) (3×250 ml) at ca. 30°. The active petroleum ether-insoluble fraction [EC_{100} (*E. histolytica* and *T. vaginalis*) (3): 200 μg/ml] was crystallized from CH_2Cl_2/petroleum ether to give orange crystals (28 g), mp 141-142° [lit. (10) 141-142°]. Microanalytical data and spectral data (ir, uv, ^1H nmr) were found to correspond with those reported for rapanone (10). Mass spectral (eims, 70 eV) data showed that the compound was principally rapanone (75.7%) [*m/z* 320 (M^+-2)] containing small amounts of embelin (18.3%) [*m/z* 292 (M^+-2)] and the higher $C_{21}H_{34}O_4$ homologue (6.0%) [*m/z* 348 (M^+-2)]. The percentage composition of the mixture was determined by the ratio of the intensities of the respective M^+-2 peaks.

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