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Note

Isolation of artemisinin (qinghaosu) and its separation from artemisitene using the Ito multilayer coil separator–extractor and isolation of arteannuin B

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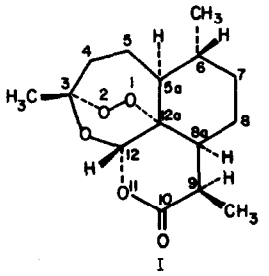
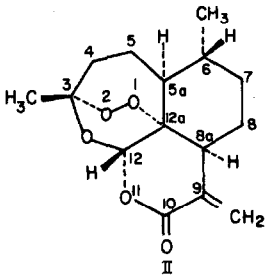
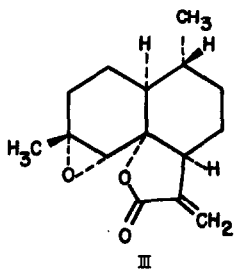
The isolation of the known endoperoxide artemisinin (qinghaosu, I), a promising antimalarial agent[1], from *Artemisia annua* collected in the United States has been reported[2]. Recently, we isolated from *A. annua* a new sesquiterpene endoperoxide, artemisitene (II), which was separated from artemisinin by repeated normal-phase high-performance liquid chromatography (HPLC)[3]. Another sesquiterpene found in *A. annua*, arteannuin B (III), may be of biosynthetic importance in the production of artemisinin. This compound has been identified[4], but the procedure for obtaining it from the plant has not been published.



I



II



III

Isolation of artemisinin involves chromatography of the crude plant extract on silica gel with chloroform–ethyl acetate as eluent[2]. Because artemisinin and artemisitene co-chromatograph on silica gel and co-crystallize from widely different solvent systems, crystalline samples of artemisinin frequently contain as much as 10% of artemisitene. The difficulty and expense of this purification procedure for large-scale production of artemisinin, as well as the contamination of the material so obtained, led us to search for an alternative isolation procedure.

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We describe here: the use of the Ito multilayer coil separator–extractor to achieve the isolation of artemisinin and arteannuin B from *A. annua* extracts, the use of this apparatus to separate artemisinin from artemisitene and the isolation of arteannuin B from *A. annua* by chromatography on silica gel.

EXPERIMENTAL AND RESULTS

Apparatus

An Ito multilayer coil separator–extractor (P.C. Inc., Potomac, MD, U.S.A.) was equipped with a No. 10 gauge (2.6 mm I.D.) coil, volume 400 ml. The stationary phase was isooctane–ethyl acetate (7:3), and the mobile phase consisted of methanol–water (6:4). Stationary and mobile phases were equilibrated before use.

Isolation of artemisinin and arteannuin B from A. annua

The top half of *A. annua* plants, grown in Silver Spring, MD, U.S.A., were harvested in August 1985. The center stalk was removed, and the plant material, which was air dried and coarsely ground (122.7 g), was extracted with light petroleum as previously described[2]. HPLC assay with electrochemical detection (ED)[5] of the extract showed the presence of 150 mg (0.12% yield) of artemisinin. The crude extract was taken up in *ca.* 15 ml of a 1:1 mixture of mobile and stationary phases for injection.

The coil was filled with stationary phase, then the entire samples was introduced at once. The coil was rotated at 800 rpm, and mobile phase was pumped through at *ca.* 2 ml/min. Those fractions containing artemisinin (elution volume *ca.* 400 ml), as determined by HPLC–ED, were combined, and the methanol was removed under reduced pressure at 35°C. The remaining aqueous suspension was extracted with diethyl ether (4 ×), the ether solution was dried ($MgSO_4$) and the diethyl ether evaporated. Crystallization of the residue from 4 ml of cyclohexane afforded 123 mg (82% recovery) of artemisinin which, after a second recrystallization, melted at 153–154°C (*cf.* ref. 6: m.p. 156–157°C); the IR spectrum was identical with that of pure material.

Earlier fractions (elution volume 200 ml) contained arteannuin B as determined by HPLC (UV detection at 220 nm), as well as artemisitene (ED). They were combined and worked up as for artemisinin (see above). Triturating the oily residue with ether afforded 83 mg of crystalline arteannuin B, m.p. 148.5–150°C (*cf.* ref. 4: m.p. 152°C) with IR and NMR spectra identical with those reported[4]. Artemisitene remained in the mother liquor, from which it could be isolated in trace amounts (< 1 mg) by silica gel chromatography, eluting with 5% ethyl acetate in chloroform.

Separation of artemisinin from artemisitene

The apparatus and conditions were identical to those described above. The crystalline sample of artemisinin (590 mg) containing *ca.* 10% of artemisitene was introduced in 8 ml of ethyl acetate–methanol–isooctane (3:3:2). When the mobile phase began to be eluted (after 50 ml), fractions of 7 ml each were collected. Artemisitene was eluted in fractions 14–22. These were combined, the organic solvent was removed at 35°C, and the aqueous residue was extracted with methylene chloride (3 ×). After drying ($MgSO_4$), the solvent was evaporated, and the residue recrystallized

from diethyl ether–light petroleum to yield 54 mg (91% recovery) of artemisitene, m.p. 163.5–164.5°C (*cf.* ref. 3: m.p. 161–162°C). Artemisinin was eluted in fractions 35–55. After workup as for artemisitene (see above) recrystallization from diethyl ether–light petroleum afforded 438 mg (82% recovery) of artemisinin, m.p. 150–152°C.

Isolation of arteannuin B by column chromatography

Dried *A. annua* (West Virginia, October 1984, 40.2 kg) was extracted by covering the plant material with Freon TF (1,1,2-trichloro-1,2,2-trifluoroethane) in two 55-gallon drums for 165 h. After evaporation of the solvent, 1.22 kg of residue remained. This was taken up in methylene chloride (450 ml), and waxes (654 g), precipitated by addition of 4 l of acetonitrile, were removed by filtration. Evaporation of solvent left 350 g of residue, which was adsorbed on 400 g of silica gel (E. Merck, silica gel 60). This was placed on top of a column containing 5.25 kg of silica gel, and the column was eluted with methylene chloride–ethyl acetate (25:1). Fractions containing arteannuin B (elution volume 19–23 l) were combined. After removal of solvents, arteannuin B (8.4 g) was obtained by crystallization of the residue (39 g) from cyclohexane. A second extraction (46 h) followed by chromatography gave an additional 2.7 g of arteannuin B, for a total yield of 0.027% of material identical with that described above (*Isolation of artemisinin and arteannuin B from A. annua*), m.p. 152–154°C.

DISCUSSION

Contamination of artemisinin with varying amounts of artemisitene is a complication and a potential hindrance in its development as an antimalarial drug. Our ability to effect a wide separation of artemisinin and artemisitene on the Ito apparatus means that artemisinin can be obtained free of artemisitene on a reasonably large scale and in a short time. Moreover, artemisitene can be isolated in sufficient quantity for further chemical transformations. For this separation of artemisinin from artemisitene, the Ito apparatus is superior to HPLC because considerably larger amounts can be injected and because the recovery is better than that obtained by normal-phase HPLC.

The stationary phase mixture used in the Ito multilayer coil separator–extractor is readily recovered by flash distillation on a rotary evaporator. The mobile phase, consisting of methanol and water, is the only material consumed in this isolation of artemisinin and arteannuin B from extracts of *A. annua*. Both aspects make this a very economical procedure. We are now trying to develop methods that would allow the isolation of multi-gram quantities of artemisinin from plant extract using the Ito apparatus.

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