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Antitumour activity of amooranin from *Amoora rohituka* stem bark

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The *in vivo* antitumour activity of amooranin was determined using N-nitrosomethyl urea-induced Sprague–Dawley rats mammary adenocarcinoma. Intraperitoneal administration of the compound at a dose of 10 or 20 mg/kg/day prolonged the mean survival time of the animals and significantly reduced tumour sizes.

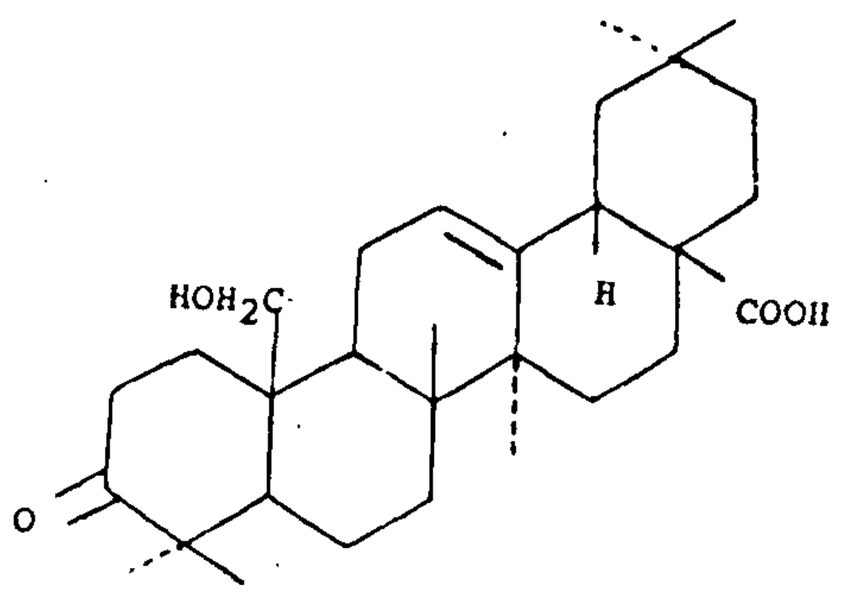
AMOORANIN is a triterpene acid with a novel skeleton isolated from *Amoora rohituka*, which is a medicinal plant used in the diseases of spleen, liver, tumours, abdomen and in rheumatism[1]. The stem bark was collected from Varanasi, India. The botanical identification of *Amoora rohituka* was confirmed by Suman, BHU, Varanasi, India (A voucher specimen is available in the herbarium).

The bark (2.1 kg) was washed, air-dried and ground into a coarse powder. It was extracted in a soxhlet with ethanol for 48 h. The ethanolic extract was vacuum evaporated to give a black residue (240 g). This residue was diluted with water and fractioned successively with petroleum ether, ethyl acetate and *n*-butanol to give petroleum ether, ethyl acetate, *n*-butanol and water-soluble fractions. The petroleum ether, ethyl acetate and *n*-butanol fractions were concentrated *in vacuo*, dried to constant weight at 37°C. The ethyl acetate extractive (50 g) was directly chromatographed on a silica gel column using ethyl acetate as eluant. The ethyl acetate fraction was chromatographed on a silica gel column, resulting in the isolation of a compound provisionally assigned as AR-II. It produced white crystal (200 mg) on crystallization from methanol.

White crystals, m.p. 276–280°C, IR (KBr) Vmax cm^{-1}, 3433 (OH), 2854 (C=O), 1689 (C=C). 1585, 1383, 1032; EIMS: M^+ 470, 440, 426, 409, 396, 379, 248, 223, 203, 191 and 133; ^{1}H-NMR (600 MHz $CDCl_3$): δ0.75 (3H, s, 26-Me), 0.91 (3H, s, 29-Me), 0.93 (3H, s, 30-Me), 0.98 (3H, s, 24-Me), 1.13 (3H, s, 23-Me), 1.25 (3H, s, 27-Me), 2.44 (2H, m, C2-H), 2.82 and 2.88 (1H, dd, C18-H), 3.46 and 3.96 (1H each, pair of AB doublets, CH_2OH), 5.3 (1H, t, C12–H).

A sample of AR-II (30 mg) treated with ethereal diazomethane yielded a methyl ester (20 mg). The methyl ester (20 mg) was treated with Ac_2O/pyridine (1 : 1) at room temperature overnight. Workup in the usual way afforded methyl ester acetate AR-IIa; amorphous powder (15 mg); m.p. 161–165°C; ^{1}H-NMR (400 MHz $CDCl_3$): δ0.75 (3H, s, 26-Me), 0.95 (3H, s, 29-Me), 0.93 (3H, s, 30-Me), 0.98 (3H, s, 24-Me), 1.12 (3H, s, 23-Me), 1.23 (3H, s, 27-Me), 2.0, (3H, s, O–C–CH_3), 3.6 (3H, s, COOMe), 3.9 and 4.6 (1H each, pair of AB doublets, CH_2OH), 5.26 (1H, t, C12–H).

Treatment of methyl ester of AR-II (13 mg) with sodium borohydride furnished a diol AR-IIb amorphous powder (10 mg); m.p. 200–203°C. ^{1}H-NMR (600 MHz $CDCl_3$): δ0.75 (3H, s, 26-Me), 0.91 (3H, s, 29-Me), 0.93 (3H, s, 30-Me), 0.98 (3H, s, 24-Me), 1.12 (3H, s, 23-Me), 1.23 (3H, s, 27-Me), 2.82 and 2.88 (1H, dd, C18–11), 3.35 and 4.15 (1H each, pair of AB doublets, CH_2OH), 3.42 (1H, m, C3–H), 3.62 (3H, s, COOME) and 5.26 (1H, t, C12–H).



For *in vivo* studies, N-nitrosomethyl urea-induced primary breast adenocarcinomas in Sprague–Dawley rats were transplanted sc beneath the lower thoracic mammary fat pads into female Sprague–Dawley rats 60–90 days old and divided into four groups of six animals each. One group was kept as control and the other three for treatment. On day 50 after tumour transplantation, and when the tumour volume was 50–150 mm^3, the treatment by intraperitoneal administration of amooranin or tamoxifen started in doses of 10 mg/kg, 20 mg/kg or 0.5 mg/kg once every day for three weeks. Tumours

Table 1. Effect of amooranin against the growth of mammary adenocarcinoma in Sprague–Dawley rats

Drug	Dose (mg/kg)	Maximal T/C% (day)*	Number of rats survived after tumour transplantation		Mean survival time (MST) ± S.D.	% ILS†
			90 days	125 days		
PBS	—	—	6/6	0/6	96.00 ± 5.83	—
	20	56.0 (16)	6/6	1/6	120.33 ± 7.53	25.34
	10	60.1 (14)	6/6	1/6	107.00 ± 8.89	11.46
Tamoxifen	0.5	17.3 (19)	6/6	6/6	164.00 ± 9.80	70.83

The treatment was started on day 50 after tumour transplantation and when the tumour volume was 50–150 mm^3.

*The day at which difference between T and C was maximal. All values were significantly different ($P < 0.01$) from controls.

†Percent increased life span: [(Mean survival time of treated group/mean survival time of control group) × 100] – 100.

were measured by Caliper measurement every 3–4 days and volumes were calculated by multiplying length × width × height × 0.5. Inclusion of height in the calculation improved the accuracy of measurements. Survival was recorded daily for 172 days. Antitumour activity was evaluated from mean survival times and by calculation of the T/C (tumour size of treated rats divided by tumour size of control rats)[2].

The results of this study are shown in Table 1. Control rats treated with PBS had a mean survival time of 96 days, while in rats treated with 10 or 20 mg of amooranin per kg, the mean survival time was 107 or 120.33 days with % ILS of 11.46 or 25.34, respectively. The rats treated with tamoxifen at 0.5 mg/kg had a mean survival of 164 days with % ILS of 70.83. Amooranin at a dose of 10 or 20 mg/kg achieved 60.10% or 56% T/C for tumour size ($P < 0.01$). Tamoxifen at the dose of 0.5 mg/kg achieved 17.3% T/C for tumour size. Although the first treatment with amooranin at 10 mg/kg usually caused a tumour growth delay from day 7, this effect was not longlasting and the tumours soon reached sizes comparable to non-treated groups after the day 24, leading to death. The effects of the amooranin and tamoxifen at 20 mg/kg and 0.5 mg/kg were studied. Observed tumour growth delay at these doses could be attributed to the better antitumour activity. The tumour growth delay was longlasting; the tumour did not reach sizes comparable with the non-treated tumours.

Although the *in vivo* activity seen with amooranin was inferior to that of tamoxifen, amooranin has some antineoplastic activity against N-nitrosomethyl urea induced Sprague–Dawley rats mammary adenocarcinoma. The mechanism through which amooranin induces its biological activity is not known.

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Flowering process in generative buds of woody perennials: An experimental approach

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The timing and extent of reproductive determination of generative buds of woody perennials not conceivable by conventional anatomical methods can be obtained by using the bud culture technique. Potentially generative buds are taken for *in vitro* culture at sequential stages of development through the annual cycle and an imagery of the change in their morphogenetic status visualized by examining the sprouts produced. The traditional technique of microscopic examination is only a superficial indicator of their reproductive status as the ripe-to-flower state may precede or succeed the histologically discernible stages.

FOWERING in woody perennials, particularly the temperate ones, is an extended process with a long time lapse between the initiation of potentially generative buds on the branches and subsequent anthesis and bud burst months later. The basic questions regarding the time of reproductive determination and the extent of irre-