Iridoids: A New Class of Leishmanicidal Agents from Nyctanthes arbortristis

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IRIDOIDS: A NEW CLASS OF LEISHMANICIDAL AGENTS FROM *NYCTANTHES ARBORTRISTIS*[1]

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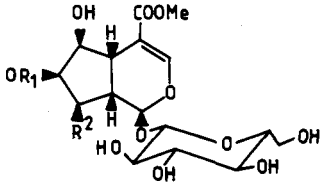
ABSTRACT.—Iridoid glucosides (arbortristosides A [**1**], B [**2**], C [**3**], and 6β-hydroxyloganin [**4**]) isolated from the traditional plant *Nyctanthes arbortristis* show antileishmanial activity in both in vitro (against amastigotes in macrophage cultures) and in vivo (in hamsters) test systems.

The wide distribution of visceral leishmaniasis (kala-azar) in the tropical regions, particularly in the Indian subcontinent, results in large scale morbidity and mortality each year. Among the protozoal diseases it can be ranked next to malaria in difficulty of treatment and control. The drugs in current use, such as sodium stibogluconate and pentamidine, suffer from toxicity, nonresponsiveness, and drug resistance. To develop effective, nontoxic oral drugs in order to combat the deadly disease remains a desirable objective.

In our research program of development of chemotherapeutic agents from Indian medicinal plants, we have encountered promising antileishmanial activity in *Nyctanthes arbortristis* L. (Oleaceae), a plant widely used in traditional remedies and folkloric medicines in India (1). Bioassay-linked extraction and fractionation of the seed extract showed the *n*-BuOH fraction to be most active. Further purification of the *n*-BuOH fraction resulted in the isolation of iridoid glucosides arbortristosides A [**1**], B [**2**], and C [**3**] (2) and 6β-hydroxyloganin [**4**] (3). The iridoid glucosides showed high order of activity against *Leishmania donovani* amastigotes in vitro and in vivo. The method used for antileishmanial testing was as described earlier (4,5). Iridoids, a group of highly functionalized monoterpenoid glycosides with the cyclopenta[*c*]pyran skeleton, have long been studied as biosynthetic precursors of indole alkaloids; however, lately they have shown various activities such as antimicrobial (6), antitumor (7), etc. Our findings of antileishmanial activity in this class of compounds may encourage further exploration of their use in the development of antileishmanial drugs.



1 R^1=*p*-methoxy cinnamoyl, R^2=Me
2 R^1=caffeoyl, R^2=CH_2OH
3 R^1=coumaroyl, R^2=Me
4 R^1=H, R^2=Me

Lethal action of all iridoids (arbortristosides A, B, C and 6β-hydroxyloganin) against amastigotes in macrophage cultures in an in vitro system was observed as a gradual decrease (i.e., inhibition) in the percentage infection as well as average number of amastigotes per macrophage with the increase of concentration (30–100 μg/ml, Table 1). In the in vivo antileishmanial testing in hamsters, using the *L. donovani* screening model, the *n*-BuOH fraction was found to possess maximum activity (Guru *et al.*, unpublished data). Activity obtained on day 28 was more than that on day 7. The pure compounds isolated from the *n*-BuOH fraction were administered ip at a dose of 10 mg/kg given for 5 days (10 mg/kg is

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TABLE 1. In Vitro Antileishmanial Activity of Iridoid Glucosides from *Nyctanthes arbortristis*.

Drug	Dose (μg/ml)	Inhibition of infected macrophages (%)	Inhibition of average number of amastigote per infected macrophage (%)
Arbortristoside A [**1**]	30	7.13 ± 5.83	45.83 ± 7.21
	100	30.24 ± 3.46	64.58 ± 3.60
Arbortristoside B [**2**]	30	28.13 ± 2.21	25.12 ± 7.11
	60	41.74 ± 2.91	47.34 ± 9.12
Arbortristoside C [**3**]	30	39.91 ± 3.38	67.43 ± 4.02
	60	toxic	toxic
6β-Hydroxyloganin [**4**]	30	42.22 ± 1.69	65.77 ± 4.56
	60	toxic	toxic
Sodium stibogluconate	30	80	80
	100	100	100

the minimum effective dose of sodium stibogluconate). Arbortristoside A showed significant antileishmanial activity of 79.68 ± 21.68%. Oral administration of this compound at 100 mg/kg/day × 5 showed 57% inhibition. Sodium stibogluconate used as positive control and tested at 10 mg/kg/day × 5 (ip) produced 71–75% inhibition in this test system (Table 2).

This shows that iridoid glycosides of Indian medicinal plant *N. arbortristis* possess promising action against leishmania infection in experimental animals. Synthetic analogues of the compounds are being prepared in order to study the structure-activity relationships and their possible mode of action.

EXPERIMENTAL

PARASITE.—*Leishmania donovani* (strain, HOM/IN/80/Dd8) was originally isolated from a human kala-azar patient from Bihar, India in 1979, and subsequently obtained in 1981, from Imperial College, London, through the kind courtesy of Prof. P.C.C. Garnham. Since then the strain has been maintained in the laboratory through serial passage (amastigote to amastigote) in golden hamsters and has been well adapted to this animal species (4,5).

HOST.—Male golden hamsters (*Mesocricetus auratus*), 40–45 g, served as experimental host for in vivo antileishmanial screening. The animals were bred and conventionally reared in the Institute's animal house. These were maintained in temperature-regulated rooms and were provided with standard rodent diet.

Cotton rats (*Sigmodon hispidus*) of either sex, 50–60 g, were used for collecting peritoneal macrophages, which were used for testing antileishmanial activity in vitro.

PREPARATION OF EXTRACT.—*General Methods.*—Details of general procedures and chromatographic techniques were provided in our earlier papers (3,8).

Plant Material.—Seeds of *N. arbortristis* were collected from Lucknow (Uttar Pradesh) in January 1985. A voucher specimen (1176 CDRI) is deposited in the Herbarium of Medicinal Plants of the Central Drug Research Institute, Lucknow.

Extraction.—The seeds of *N. arbortristis* (7 kg)

TABLE 2. In Vivo Antileishmanial Activity of Iridoid Glucosides of *Nyctanthes arbortristis*.

Drug[a]	Inhibition post treatment (%)		Survival of animals (days)	
	Day 7	Day 28	Experimental	Control
Arbortristoside A	18.23	79.68 ± 21.68	38	<32
Arbortristoside B	No inhibition	No inhibition	59	<32
Arbortristoside C	No inhibition	—	<28	<32
6β-hydroxyloganin	30.81	—	<28	<32
Sodium stibogluconate	71.9	93.51	<30	<35

[a]Dose 10 mg/kg/day × 5 (ip).

were exhaustively extracted with 50% EtOH (5 × 1.5 liters) at room temperature. The combined extracts were evaporated in vacuo below 45° to give a residue (850 g). The concentrated EtOH extract (400 g) on subsequent fractionation with hexane, $CHCl_3$, and *n*-BuOH gave 10-g, 60-g, and 140-g fractions, respectively.

ISOLATION OF ARBORTRISTOSIDE A.—The concentrated *n*-BuOH fraction (70 g) was chromatographed on a Si gel (1.5 kg) column and eluted with EtOAc and EtOAc (saturated with H_2O)/MeOH with increasing content of MeOH; this afforded fraction A (8 g) and fraction B (4.6 g). Fraction A was rechromatographed on Si gel (250 g) and eluted with $CHCl_3$/MeOH; 7% MeOH/$CHCl_3$ yielded arbortristoside A, which was crystallized from EtOH as white needles (4 g), mp 220–222° (3).

SCREENING TECHNIQUES.—*In vivo*.—The test method used has been described in detail earlier (4,5). The amastigotes isolated from infected spleen (9) of hamster were inoculated (0.1 ml of Locke's solution with 1×10^7 amastigotes per hamster) intracardially to healthy hamsters. The infection establishes itself within 20–25 days and the hamsters also gain weight (80–100 g) and can now sustain the repeated spleen biopsies which are performed before as well as after treatment. Animals with +2 infection (11 to 50 amastigotes per 100 spleen cell nuclei) were used for screening. The drug solution was prepared by grinding the accurately weighed extract, fraction, or pure compound with distilled H_2O. In case of insoluble compounds, they were suspended in H_2O or EtOH with 1–2 drops of Tween-80, and further dilutions were made in H_2O. Infected hamsters were treated with different doses of test materials administered ip for 5 consecutive days, starting 3–4 days after pretreatment spleen biopsies that were done to assess the initial parasite count. Subsequently, spleen biopsies were performed on days 7 and 28 post-treatment. Spleen dab smears, fixed in absolute MeOH and stained with Giemsa, of treated and untreated animals were assessed for parasite burden (number of amastigotes/100 cell nuclei). The efficacy was expressed in terms of percentage inhibition of multiplication of amastigotes in spleen using the following formulae (4).

$$\% \text{ Inhibition} = 100 - \frac{\text{actual number of amastigotes in treated animals} \times 100}{\text{initial number of amastigotes in treated animals} \times \text{Increase in untreated control}}$$

Three or four animals were used for each dose of the test material, and data of two or more replicates were pooled for calculation of mean percentage inhibition.

In vitro.—The test method used for assessing antileishmanial activity was similar to that described by Bhatnagar *et al.* (5). The cotton rat (*S. hispidus*) peritoneal macrophages infected with promastigotes of *L. donovani* in Leighton tubes using RPMI-1640 medium with 10% fetal bovine serum were used. The various details of step-by-step procedure used for testing in vitro antileishmanial action were also similar to those of Bhatnagar *et al.* (5). The following formulae were also used for calculating the percentage inhibition of infection.

$$\% \text{ Inhibition} = 100 - \frac{\% \text{ macrophages infected in treated cultures} \times 100}{\text{mean } \% \text{ macrophages infected in untreated controls}}$$

and

$$\% \text{ Inhibition} = 100 - \frac{PT \times 100}{PC},$$

where PT = average number of amastigotes/macrophages in treated tubes and PC = average number of amastigotes/macrophage in control tubes.

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LITERATURE CITED

1. "Medicinal Plants of India," ICMR Publication, India, 1987, Vol. 2, p. 343.
2. K.K. Purushothaman, M. Venkatanarasimhan, and A. Sarada, *Phytochemistry*, **24**, 773 (1985).
3. A. Rathore, R.K. Juneja, and J.S. Tandon, *Phytochemistry*, **28**, 1913 (1989).
4. P.Y. Guru, A.K. Agarwal, U.K. Singha, A. Singhal, and C.M. Gupta, *FEBS Lett.*, **245** (12), 204 (1989).
5. S. Bhatnagar, P.Y. Guru, J.C. Katiyar, R. Srivastava, A. Mukherjee, M.S. Akhtar, M. Seth, and A.P. Bhaduri, *Indian J. Med. Res.*, **89**, 439 (1989).
6. K. Ishiguro, M. Yamaki, and S. Takagi, *Yakugaku Zasshi*, **102**, 755 (1982); *Chem. Abstr.*, **99**, 142349q (1983).
7. K. Ishiguro, M. Yamaki, S. Takagi, Y. Ikeda, K. Kawakami, K. Ito, and T. Nose, *Chem. Pharm. Bull.*, **34**, 2375 (1986).
8. A. Rathore, V. Srivastava, K.C. Srivastava, and J.S. Tandon, *Phytochemistry*, **29** (6), 1917 (1990).
9. L.A. Stauber, E.M. Franchino, and J. Grun, *J. Protozool.*, **5**, 269 (1958).

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