

CYPERENOIC ACID AND (–)-HARDWICKIIC ACID FROM THE CHLOROFORM EXTRACT OF THE ROOTS OF *CROTON AROMATICUS* : ISOLATION AND INSECTICIDAL PROPERTIES

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Abstract : Cyperenoic acid and (–)-hardwickiic acid have been isolated from the chloroform extract of the roots of *Croton aromaticus* L. Both compounds displayed moderate insecticidal activity against the groundnut aphid, *Aphis craccivora*. Comparison of antifungal and insecticidal properties exhibited by the extracts of *C. aromaticus* L. and *C. lacciferus* L. corroborates previous conclusions of chemotaxonomic studies that the two taxa be maintained as two distinct species.

1. Introduction

Pesticides are frequently used in agriculture for the control of pathogens and insects. The continuous application of synthetic pesticides, however, brings about human health hazards, environmental pollution, lack of species-specificity and development of pesticide resistant strains. Investigation of new sources for pesticides is important with a view to developing agrochemicals having specific properties. Compounds of plant origin may have less harmful effects on mammals than the synthetic pesticides.

The second largest genus of Euphorbiaceae, the spurge family is that of *Croton*. Some *Croton* species have shown insecticidal properties e.g. extracts of *C. glandulosus* L. and *G. tiglium* L. have displayed insecticidal activity against boll weevils and house flies, respectively.^{1,3} The extracts of *C. aromaticus* L. and *C. lacciferus* L. have caused toxic effects to the cowpea bruchid, *Callosobruchus chinensis*^{1,8} and the groundnut aphid, *Aphis craccivora*.⁸ A new clerodane derivative having significant aphidicidal activity has been isolated from *C. lacciferus*.⁸ In a preliminary communication,⁵ it has been reported that (–)-hardwickiic acid (1), the major constituent of the light petroleum (60–80°C) extract of the roots of *C. aromaticus*,⁴ also contain some aphidicidal properties. The present study describes

the isolation of (1) and cyperenoic acid (2) from the chloroform extract of the roots of *C. aromaticus* and evaluation of the insecticidal activity of 2 against *A. craccivora*.

C. aromaticus and *C. lacciferus* which find application in traditional agriculture and ethnomedicine,^{1,4} are commonly known as "keppetiya" in Sinhala and "theppaddi" in Tamil.⁹ The two taxa have somewhat similar morphological characters and consequently Trimen has classified them as varieties of a single species.²⁰ A recent chemotaxonomic study,⁴ also supports the contention that they are two distinct species.^{1,14} In this paper the question of taxonomy of *C. aromaticus* and *C. lacciferus* is also addressed in terms of their biological activity, particularly antifungal and insecticidal properties.

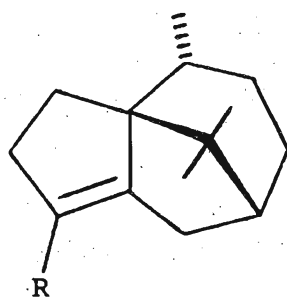
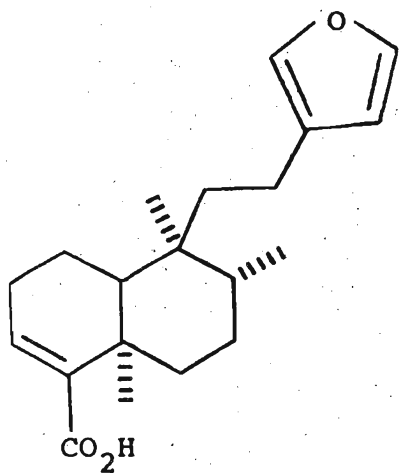
2. Results and Discussion

2.1 Isolation of compounds

The air-dried and powdered roots of *C. aromaticus* were sequentially extracted with light petroleum (60–80°C), chloroform and methanol under reflux conditions. Chromatographic fractionation of the light petroleum extract gave β -amyrin and (–)-hardwickiic acid (1) in $1.2 \times 10^{-2}\%$ and 0.8% yield, respectively.⁴

A part of the chloroform extract was chromatographed over silica gel and elution with 2% ethyl acetate in benzene afforded 1 in 0.24% yield. Compound 1 was identified by comparison with an authentic sample⁴ (m.p., TLC, IR and ¹H-NMR).

Further elution of the column with 5% ethyl acetate in benzene followed by flash chromatography and thin layer chromatography (TLC) gave a white crystalline compound (2) m.p. 163–164°C, in $2.55 \times 10^{-3}\%$ yield. The LiAlH₄ reduction of 2 gave the corresponding alcohol (3) in 91% yield. The Physical data of 2 and 3 closely resembled those reported for cyperenoic acid and cyperenol, respectively,^{12,16,17,19,21} and the structures were confirmed by a single crystal X-ray study of 2 (Figure 1). The atomic coordinates of 2 have been reported in an independent X-ray analysis recently.¹⁰ The compound 2 has been isolated from *C. crassifolius* Geisel¹⁰ and *Sandwithia guyanensis* Lanj. (Euphorbiaceae).¹²



2 R = CO₂H

3 R = CH₂OH

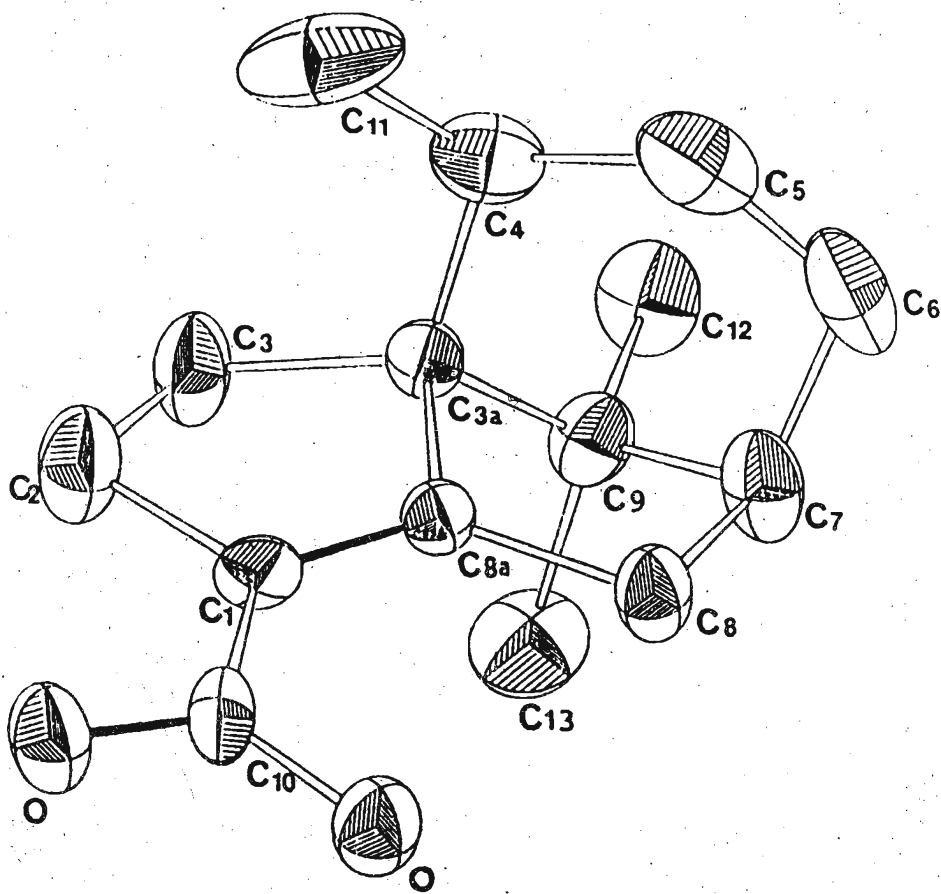


Figure 1. The X-ray perspective (ORTEP) diagram of cyperenoic acid (2).

2.2 Insecticidal properties of the compounds

The insecticidal activity of cyperenoic acid (2) and β -amyryn was examined, using the microapplicator method,¹¹ on aphids which had been reared on the leaves of *Vigna unguiculata* L. (var. *Bushitavo*) in the laboratory. The compound 2 and β -amyryn at a dose of 0.5 μ g/insect caused 54% ($P < 0.05$) and 39% mortality, respectively, in female aphids after 24 h. (–)-Hardwickeic acid (1) exhibited higher toxic effects (62% mortality, $P < 0.005$) towards aphids under identical conditions.⁵

2.3 Taxonomy of *C. aromaticus* and *C. lacciferus* in relation to their chemical constituents and biological properties

The steam distillate from the leaves of *C. lacciferus* recorded a much higher yield than that from *C. aromaticus* (Table 1).

Table 1. Antifungal activity (against *Cladosporium cladosporioides*) and insecticidal activity (against *Aphis craccivora*) of the extractives of *Croton aromaticus* and *C. lacciferus*

Plant and Plant part (kg) ^a	Extractive (g) ^b	Antifungal activity ^c R _f (CHCl ₃) ^d	Insecticidal activity ^e (percentage mortality)	
			24 HAT	48HAT ^f
<i>C. aromaticus</i>				
Roots (1.8)	petroleum (110)	—	33	78 ^g
	chloroform (33)	—	28	72 ^g
	methanol (55)	—	39	51 ^g
Leaves (1.8)	steam distillate	0.2	29	73 ^g
	(1.1)	0.4		
		0.6		
<i>C. lacciferus</i>				
Roots (4.5)	petroleum (67)	—	85 ⁱ	89 ^h
	chloroform (42)	0.4	42 ^g	73 ^g
	methanol (54)		07	48
Leaves (2.85)	steam distillate	0.4	05	34
	(14)	0.6		

^a weight of the plant material in kg; ^b weight of the extractive in kg; ^c reference 5; ^d R_f of the zone of inhibition on the TLC-bioassay plate; ^e reference 2; ^f hours after treatment; ^g $P < 0.05$; ^h $P < 0.01$; ⁱ $P < 0.001$.

The composition of each distillate differed from one another significantly as evident from the GC–MS analysis of the leaf volatiles.⁶ The roots of *C. lacciferus* afforded a range of kauranoids and oleananes^{2,7} which were not detected in *C. aromaticus*. (–)-Hardwickiic acid (1) was isolated in excess of 1% yield from the dried roots of *C. aromaticus*; this is a high yield for a secondary metabolite from a plant source. In contrast, the compound 1 was almost non-existent in the root extracts of *C. lacciferus*.⁴ The direct comparison by high performance liquid chromatography of leaf and root extracts which had been prepared under comparable conditions, further revealed that each plant has a distinct chemical composition.⁴

The antifungal activity of the leaf volatiles and the root extracts was examined against *Cladosporium cladosporioides*³ using the TLC–bioassay technique.¹⁵ The results are given in Table 1. The chloroform extract of the roots of *C. lacciferus* displayed a zone of inhibition in the TLC–*Cladosporium*–bioassay plate, and the corresponding active compound was identified as 2,6–dimethoxybenzoquinone.³ None of the root extracts of *C. aromaticus* showed antifungal activity. The zone of inhibition at R_f 0.2 observed for the leaf volatiles of *C. aromaticus* was absent in the case of *C. lacciferus*.

The steam distillate of *C. aromaticus* caused higher toxic effects to aphids than that of *C. lacciferus* (Table 1). The petroleum extract of the roots of *C. lacciferus* displayed considerably high aphidicidal activity compared to that of *C. aromaticus*. It is also noted that the root extracts showed bioactivities, distinctive of each plant, against the cowpea bruchid, *Callosobruchus chinensis* reared on *Vigna radiata* seeds in the laboratory.¹⁸

The observations made with respect to the chemical constitutions and bioactivities of the two taxa strongly support the contention that *C. aromaticus* and *C. lacciferus* be maintained as two distinct species.^{1,14}

3. Experimental

3.1 Preparation of plant extracts

Plant samples of *C. aromaticus* were collected near Dambulla and a voucher specimen has been deposited in the University herbarium. The crushed dried roots (4.3 kg) were sequentially and exhaustively extracted with light petroleum (b.p. 60–80°C), chloroform and methanol under reflux conditions. The removal of solvent on a rotavapor gave each extract as a brown semi-solid; the yields are given in Table 1. The preparation of leaf volatiles of *C. aromaticus* and *C. lacciferus*³ and root extracts of *C. lacciferus*^{2,7} have been described previously.

3.2 Isolation of compounds from the root extract of *C. aromaticus*

A part (25 g) of the chloroform extract was chromatographed on a silica gel (mesh 70–230, 700 g) column. Elution of this column with 2% ethyl acetate in benzene afforded a white solid (7.8 g, 0.24%), m.p. 103–104°C (*n*-hexane), (lit.^{4,5} 103–104°C). This compound was identified as (–)-hardwickiic acid (1) by comparison with an authentic sample (m.p., TLC, IR, ¹H NMR). Further elution of the column with 5% ethyl acetate in benzene followed by medium pressure chromatography (40% CH₂Cl₂ – petroleum) and TLC (0.5% MeOH–CH₂Cl₂) furnished a white crystalline compound (83 mg, 2.55 × 10⁻³%), m.p. 163–164°C (*n*-hexane) (lit.^{1,2} 162–164°C); [α]_D – 8.5° (CHCl₃, *c* 1.42) (lit.^{10,12} – 7.7°, – 18.8°). This compound was identified as cyperenoic acid (2) by comparison with reported spectral data^{16,17,19,21}, chemical conversions and X-ray analysis.

Medium pressure chromatography of the petroleum extract over silica gel (TLC grade, Merck) with 25% CH₂Cl₂ – petroleum gave β-amyrin and 1 as described previously.⁴

3.3 Reduction of cyperenoic acid (2) to cyperenol (3)

To a solution of 2 (20 mg) in Et₂O, LiAlH₄ (20 mg) was added portionwise and stirred at room temperature for 13 h. Usual work-up afforded a residue which was purified using TLC (60% CH₂Cl₂ – petroleum) to obtain 3 as a white crystalline solid (1 mg, 91%), m.p. 87–89°C (*n*-hexane), lit.¹⁶ 94°C; [α]_D – 11.8° (CHCl₃, *c* 0.9), (lit.¹⁶, – 12.1°). The physical data of 3 closely resembled those reported for cyperenol previously.¹⁶

3.4 Examination of insecticidal properties

The protocol used in the bioassay of plant extracts and pure compounds is described elsewhere.⁸ Briefly, the cultures of *Aphis craccivora* were maintained in the laboratory on one-week old potted cowpea, *Vigna unguiculata* (var. *Bushitavo*) at 25–33°C, 68–84% relative humidity and 12 h photoperiod. One-day old female aphids were used in all experiments. A solution (2000 ppm) of each pure compound was prepared by dissolving in acetone. A sample of each solution (2.5 μl, 5 ppm) was directly introduced to aphids using a microapplicator,¹¹ and the mortality was counted after 24 h. Forty to fifty insects were used for each sample. The results were analysed by means of a χ² test. Compounds 1 and 2 and β-amyrin caused 62% (P < 0.05), 54% (P < 0.05) and 39% mortality to aphids, respectively.

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References

1. ABEYWICKREMA, B.A. (1959) *Cey. J. Sci. (Bio. Sci.)*, **2** : 119.
2. BANDARA, B.M.R. & WIMALASIRI, W.R. (1988) *Phytochemistry*, **27** : 225.
3. BANDARA, B.M.R., WIMALASIRI, W.R., ADIKARAM, N.K.B. & BALASUBRAMANIAM, S. (1986–87) *Cey. J. Sci. (Bio. Sci.)* **19** & **20** : 12.
4. BANDARA, B.M.R., WIMALASIRI, W.R. & BALASUBRAMANIAM, S. (1988) *J. Natn. Sci. Coun. Sri Lanka*, **16** : 87.
5. BANDARA, B.M.R., WIMALASIRI, W.R. & BANDARA, K.A.N.P. (1987) *Planta Medica*, **575**.
6. BANDARA, B.M.R., WIMALASIRI, W.R., HUSSEIN, R. & REISCH, R., in preparation.
7. BANDARA, B.M.R., WIMALASIRI, W.R. & MACLEOD, J.K. (1988) *Phytochemistry* **27** : 869.
8. BANDARA, K.A.N.P., BANDARA, B.M.R. & WIMALASIRI, W.R. (in preparation)
9. BANDARANAYAKE, W.M., SULTANBAWA, M.U.S., WEERASEKERA, S.C. & BALASUBRAMANIAM, S. (1974) *Sri Lanka Forester*, **11** : 67.
10. BOONYARATAVEJ, S., ROENGSUMRAN, S. BATES, R.B., ORTEGA, R.B. & PETSON, A. (1988) *J. Nat. Prod.* **51** : 769.
11. HENRICHS, E.A., CHELLIAH, S., VALENCIA, S.L., ARCEO, M.B.A., FLABELLAR, L.T., AQUINO, G.B. & PICKIN, S. (1981) *Manual for Testing Insecticides on Rice*, p. 13, Philippines, IRRI.
12. JACOBS, H., LACHMANSING, S.S., RAMDAYAL, F., MCLEAN, S., PUZZOLI, F.V. & REYNOLDS, W.F. (1987) *J. Nat. Prod.* **50** : 835.
13. JACOBSON, M. (1975) *Insecticides from Plants. A review of the literature 1954–1971*, Agriculture Handbook, No. 461, p. 29, Washington DC : Agriculture Research Service, USDA.
14. JAYAWEERA, D.M.A. (1980) *Medicinal Plants Used in Ceylon, Part 2*, p. 197, Colombo : The National Science Council of Sri Lanka.
15. KLARMAN, W.L. & STANFORD, J.B. (1968) *Life Science* **7** : 1095.
16. NERALI, S.B. & CHAKRAVARTI, K.K. (1967) *Tetrahedron Lett.*, 2447.
17. PATT, S.L. & SCHOOLERY, J.N. (1982) *J. Magn. Reson.* **46** : 535.
18. RANSINGHE, M.A.S.K., personal communication

19. TAKANO, S. & KAWAMINAMI, S. (1988) *Phytochemistry* 27 : 1197.
20. TRIMEN, H. (1898) Handbook to the Flora of Ceylon, Part 4, p. 46.
21. WEHRLI, F.W. & WIRTHLIN, T. (1980) Interpretation of Carbon - 13 NMR Spectra, Philadelphia : Heyden.