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ROTENOIDS OF *LONCHOCARPUS SALVADORENSIS*: THEIR EFFECTIVENESS IN PROTECTING SEEDS AGAINST BRUCHID PREDATION

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Key Word Index—*Lonchocarpus salvadorensis*; Leguminosae; *Callosobruchus maculatus*; bruchids; insecticides; rotenone; degeulin; elliptone; α-toxicarol.

Abstract—Seeds of the legume *Lonchocarpus salvadorensis*, having unusually low bruchid predation, contain deguelin (0.29%), rotenone (0.22%), elliptone (0.06%) and α-toxicarol (0.003%). The relation between rotenone content and toxicity towards the generalist bruchid *Callosobruchus maculatus* is examined and quantified.

INTRODUCTION

Although there is limited information on relative insecticidal activities [1, 2] within the rotenoid class of compounds [3], little is known of their ecological value in protecting the plants which produce them from insect attack. One particular aspect that has engaged our attention is the interaction between bruchid beetles (seed predators) and the chemical defences of wild legume seeds, especially those of the rotenoid-bearing genera of the Leguminosae (*Lonchocarpus*, *Derris* and *Tephrosia*). Examination of such questions requires knowledge of the types and concentrations of rotenoids in suitable wild legume seeds, and their insecticidal activity. In this paper we report on the identification and quantification of rotenoids found in the seeds of the tropical legume tree *Lonchocarpus salvadorensis* Petter (Papilionoideae: tribe Tephroseae), together with insecticidal bioassay data concerning the bruchid *Callosobruchus maculatus*. *Lonchocarpus salvadorensis* is known to be a species which suffers from unusually low attack by indigenous bruchids in the dry forests of Costa Rica [Janzen, D. H., personal communication].

RESULTS AND DISCUSSION

Extract of the seeds was partitioned between *n*-hexane and nitromethane, leaving fats and only traces of rotenoids in the *n*-hexane layer. The nitromethane extract was investigated by high pressure liquid chromatography (HPLC) on a silica Z-pak semi-preparative column eluting with 10% ethyl acetate in hexane. The first peak (0.003% of dry seeds) was identified as α-toxicarol (**1**) [4] (^{1}H NMR, accurate mass, and comparison with a pure sample of α-toxicarol by TLC and by normal and reversed-phase HPLC) [5, 6]. The second peak corresponds to deguelin (**2**), present in much higher concentration (0.29%) (^{1}H NMR, accurate mass, and comparison with an authentic sample as before). A mixed peak containing mainly rotenone with some elliptone was then eluted. These two rotenoids were separated by reversed-phase HPLC on a C_{18}-semi-preparative column, eluting with methanol in water to give elliptone (0.06%) and rotenone (0.22%). Both were identified by ^{1}H NMR, accurate mass, and TLC and HPLC comparison against authentic pure specimens.

Preliminary tests with the bruchid beetle *C. maculatus* showed that *Lonchocarpus salvadorensis* seed was much less attractive for oviposition than cowpea (*Vigna unguiculata*), used as a control. Though in each case 90% of the eggs laid were viable, only an average of three eggs per seed were laid on *L. salvadorensis* against 30 on cowpea. First instar larval mortality was 70% at the cotyledon entry stage for the former, and no adults emerged. By contrast there was no larval mortality in the control at cotyledon entry, and 10 adults emerged per cowpea seed. Toxicity to first instar *C. maculatus* larvae entering *L. salvadorensis* seed was found to reside in the cotyledon but not in the testa; eggs laid on testa removed from *L. salvadorensis* and repositioned on cowpea cotyledons hatched and 80% of the larvae successfully penetrated the *L. salvadorensis* testa, developing normally on the cowpea cotyledon below. In contrast, when eggs were laid directly on *L. salvadorensis* cotyledon (testa removed), larval mortality was 100% at first instar and no adults emerged. For all controls in this group of preliminary experiments eggs were 80–90% viable and larval entry (through testa into cotyledon) was 80–100% successful.

To explore the insecticidal effectiveness of rotenoids further, artificial test seeds consisting of cowpea flour contained in a gelatine capsule were made up. These were fully acceptable to *C. maculatus* for oviposition, and suitable concentrations of rotenoids could be incorporated into the cowpea flour. Results are shown in Table 1. The rotenoid mixture from *L. salvadorensis*, administered at the natural concentrations in artificial seeds, totally prevents adult emergence from *C. maculatus* eggs. Indeed the major rotenoids rotenone and deguelin administered alone at their own natural concentrations are completely lethal, and even elliptone at 0.06% has some effect relative to the control. α-Toxicarol is present at too low a

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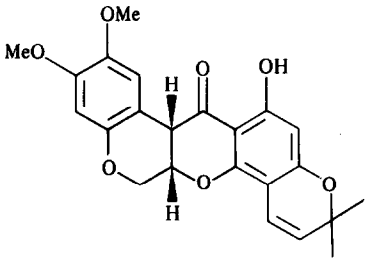

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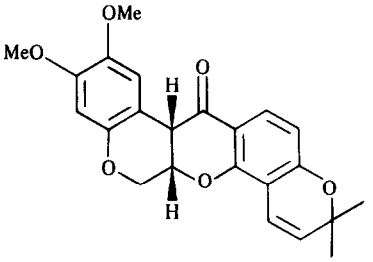

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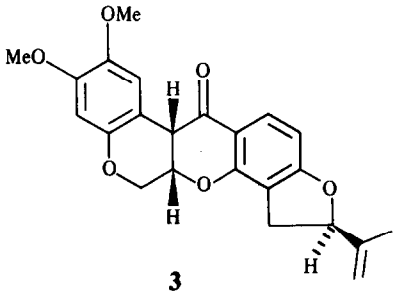

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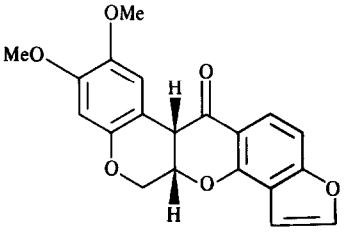

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Table 1. Bioassay of rotenoids against larvae of *Callosobruchus maculatus*, employing artificial seeds

Additive to seed	Mean emergence of adults (days)	Adults emerging per capsule
Rotenoids mixture*	—	0
Rotenone (0.29%)	—	0
Deguelin (0.22%)	—	0
Elliptone (0.06%)	28	5
α-Toxicarol (0.003%)	22	14
Control	22	14

*Mixture of the four rotenoids at the concentrations found in *Lonchocarpus salvadorensis* seeds.

Table 2. Concentration dependence of toxicity of rotenone and of deguelin towards larvae of *C. maculatus* (administration in artificial seeds)

Test rotenoid	Mean emergence of adults (days)	Adults emerged per capsule
Rotenone		
0.5%*	—	0
0.1%	—	0
0.01%	28	3
0.001%	24	10
Control	22	14
Deguelin		
0.5%	—	0
0.1%	—	0
0.01%	25	10
0.001%	24	10
Control	22	14

*In cowpea flour.

concentration to be effective. Table 2 shows that rotenone causes high mortality as a stomach poison down to 0.01% concentration and deguelin to below 0.1%.

This work establishes and quantifies the toxicity of rotenoids towards a generalist bruchid, *Callosobruchus maculatus*. Nonetheless it can be inferred from records of bruchids' host ranges on seeds likely to contain rotenoids that specialist bruchids can successfully attack such seeds. Thus rotenoid-containing seeds of the legume species *Piscidia mollis*, *Tephrosia candida* and *Pachyrrhizus erosus* [3] are each attacked by a least one specialist bruchid species [7] and it is likely that other examples exist. These specialist bruchids have probably evolved an increased ability, relative to the non-specialists, to detoxify insecticidal rotenoids [8]. A somewhat similar situation exists in the seeds of *Dioclea megacarpa* where the toxic arginine analogue canavanine is an effective chemical barrier against *C. maculatus* [9] whilst it is ineffective against the specialist *Caryedes brasiliensis* [10]. It will be of interest to identify the biochemical mechanisms involved in these specialised rotenoid detoxifications [11], and their ecological consequences.

EXPERIMENTAL

Normal-phase HPLC employed a Waters silica Z-pak column, eluting with 10% EtOAc in hexane (3 ml/min). For reversed-phase HPLC a C_{18}-semi-preparative column was used, eluting with 72.5% MeOH in H_2O (2 ml/min). TLC was on silica plates eluting with $CHCl_3$–Me_2CO–HOAc (196:3:1). ^1H NMR data were measured on a 250 MHz Bruker instrument.

Extraction of Lonchocarpus salvadorensis *seeds.* Seeds (30 g) were ground and extracted (Soxhlet) for 13 hr with Et_2O to give an oily green extract (7.71 g). Extraction was repeated (7 hr) with CH_2Cl_2 to give a green-yellow oil containing solid (0.22 g). Each extract was dissolved in *n*-hexane and re-extracted × 3 with nitromethane (volume ratios 10:1). The nitromethane extracts weighed 330 mg and 40 mg respectively and were united. The remaining *n*-hexane showed only traces of rotenoid material.

The nitromethane extractive was chromatographed using normal-phase HPLC to give α-toxicarol (1 mg, 0.003%) having normal-phase R_t 16.6 min, reversed phase R_t 12.5 min, R_f on TLC 0.55. It had $[M]^+$ at m/z 410 (Calc. for $C_{23}H_{22}O_7$, M 410); ^1H NMR ($CDCl_3$): δ6.86 (*d*, 1H, J = 0.7 Hz), 6.56 (*dd*, 1H, J = 0.6 and 10.1 Hz), 6.46 (*s*, 1H), 5.96 (*d*, 1H, J = 0.6 Hz), 5.47 (*d*, 1H, J = 10.1), 4.87 (*br t*, 1H), 4.62 (*dd*, 1H, J = 3.1 and 12.1 Hz), 4.17 (*d*, 1H, J = 12.1 Hz), 3.82 (*s*, 3H), 3.80 (*s*, 3H), ~ 3.8 (1H obscured), 1.44 (*s*, 3H), 1.37 (*s*, 3H). The second compound to be eluted was deguelin (87 mg, 0.29%), normal-phase R_t 21.3 min, reversed-phase R_t 8.5 min, R_f 0.49. It had $[M]^+$ 394.139. (Calc. for $C_{23}H_{22}O_6$, M 394.142); ^1H NMR ($CDCl_3$): δ7.75 (*d*, 1H, J = 8.7 Hz), 6.79 (*s*, 1H), 6.65 (*d*, 1H, J = 10.1 Hz), 6.46 (*s*, 1H), 6.45 (*d*, 1H, J ~ 8 Hz), 5.56 (*d*, 1H, J = 10.1 Hz), 4.92 (*br t*, 1H, J ~ 3 Hz), 4.64 (*dd*, 1H, J = 3 and 12 Hz), 4.19 (*d*, 1H, J = 12 Hz), ~ 3.8 (1H, obscured), 3.81 (*s*, 3H), 3.78 (*s*, 3H), 1.46 (*s*, 3H), 1.39 (*s*, 3H).

The third peak to be eluted had normal-phase R_t ~ 25 min and was a mixture of rotenone and elliptone. They were separated by reversed-phase HPLC. Elliptone (18 mg, 0.06%) had normal phase R_t 23.7 min, reversed-phase R_t 4.3 min, R_f 0.50. It had $[M]^+$ 352.093 (Calc. for $C_{20}H_{16}O_6$, M 352.095), ^1H NMR ($CDCl_3$): δ7.90 (*d*, 1H, J = 8.8 Hz), 7.57 (*d*, 1H, J = 2.1 Hz), 7.16 (*dd*, 1H, J = 0.9 and 8.8 Hz), 6.94 (*dd*, 1H, J = 0.9 and 2.3), 6.77 (*br s*, 1H), 6.47 (*s*, 1H), 5.10 (*br t*, 1H, J ~ 4 Hz), 4.74 (*dd*, 1H, J = 3.1 and 12.1 Hz), 4.29 (*d*, 1H, J = 12.4 Hz), 3.96 (*br d*, 1H, J ~ 5 Hz), 3.80 (*s*, 3H), 3.77 (*s*, 3H). Rotenone (65.5 mg, 0.22%) had normal-phase R_t 25.3 min. reversed-phase R_t 7.6 min, R_f 0.49. It had $[M]^+$ 394.140 (Calc. for $C_{23}H_{22}O_6$, M 394.142). ^1H NMR ($CDCl_3$): δ7.84 (*d*, 1H, J = 8.6 Hz), 6.77 (*d*, 1H, J = 0.8 Hz), 6.52 (*d*, 1H, J = 8.6 Hz), 6.46, (*s*, 1H), 5.25 (*t*, 1H, J = 8.8 Hz), 5.08 (*d*, 1H, J = 0.5 Hz), 4.94 (*m*, 2H), 4.62 (*dd*, 1H, J = 3 and 12 Hz), 4.19 (*d*, 1H, J = 12 Hz), ~ 3.8 (1H, partly obscured), 3.81 (*s*, 3H), 3.77 (*s*, 3H), 3.33 (*dd*, 1H, J = 9.8 and 15.8 Hz), 2.95 (*dd*, 1H, J = 8 and 15.8 Hz), 1.77 (*s*, 3H). ^1H NMR data were compared with those for authentic specimens and with lit data.

Bioassay of rotenoids against C. maculatus. A culture of *C. maculatus* obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria was used for all feeding experiments, maintained in a controlled environment chamber (70% r.h., 30° ± 0.5°). Artificial seeds were prepared by finely grinding cowpeas (cv. California Blackeye No. 2), pre-equilibrated at 70% r.h. for 30 days (12–14% moisture content). The flour, with or without added rotenoids, was packed into Davcap No. 2. (7 × 20 mm) hard gelatine capsules, each holding 500 mg of diet. Mated, newly-emerged female beetles (age 12–24 hr) were allowed to lay 20 eggs on each capsule. The capsules were then incubated for 40 days, monitoring larval development and adult emergence. Five capsules were made for each test chemical treatment or control, and mean emergence day and number of adults per capsule recorded.

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