

Isolation of Amorpholone, a Potent Rotenoid Insecticide from *Tephrosia candida*

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Amorpholone (6-hydroxy-6a,12a-dehydrorotenone, III) has been isolated and identified from the genus *Tephrosia* for the first time. Its structure has been confirmed by its partial synthesis from rotenone (6a,12a,4',5'-tetrahydro-2,3-dimethoxy-5'-isopropenylfuran-3',2':8,9]-6H-rotenen-12-one, I). The petroleum ether extract and the different fractions of the methanolic extract of the stems and leaves of *Tephrosia candida* along with some pure rotenone derivatives, viz., amorpholone (III), dehydrorotenone (II) (naturally obtained), and rotenonone (IV), a synthetic analogue of amorpholone, were tested for their insecticidal activity against the larvae of *Spodoptera litura* F. (armyworm), taking pure rotenone as the reference insecticide. Among the different crude extracts tested, petroleum ether showed the highest activity ($LD_{50} = 0.38 \mu\text{g/g}$ of body weight) and rotenone (I) was the most active of the pure compounds ($LD_{50} = 0.16 \mu\text{g/g}$) followed by amorpholone (III, 0.31 $\mu\text{g/g}$) and rotenonone (IV, 0.68 $\mu\text{g/g}$), while dehydrorotenone (II) was found to be completely inactive.

INTRODUCTION

The genus *Tephrosia* (family Leguminosae), estimated to contain about 300 species (Gillett et al., 1971; Willis, 1973), is reputed to possess medicinal (Chopra et al., 1956, 1969), insecticidal (Chopra et al., 1969), insect antifeeding (Lwande et al., 1985; Srimmanarayana and Rao, 1985; Bentley et al., 1987; Simmonds et al., 1990), insect growth regulatory (Chiu, 1989), and antifungal (Lwande et al., 1985) activities. Chemical investigation of several species of the genus led to the isolation of a wide array of compounds including flavonoids and rotenoids (Waterman and Khalid, 1980). *Tephrosia candida* (Roxb.) DC (common name Lashtia), an Indian species widely distributed in the tropical Himalayas from Garhwal to Khasia and Assam up to 5000 ft altitude and occasionally grown as an ornamental plant, is used as a fish poison (Chopra et al., 1956). The seeds of the plant were also reputed to possess insecticidal property (Chopra et al., 1969). Since the stems and leaves of *T. candida* were found to possess significant insecticidal and antifeeding property (Roy et al., 1987), it was, therefore, thought worthwhile to undertake further screening of this species for its insecticidal activity against the larvae of a polyphagous insect, *Spodoptera litura* F. (armyworm). Although there are only a few papers about the feeding deterrent activity of rotenone and its derivatives (Bentley et al., 1987; Nawrot et al., 1989), the insecticidal property of rotenone and related compounds is well reputed (Sarup et al., 1964, 1969; Fukami and Nakajima, 1971; Singh et al., 1975). An attempt has, therefore, been made to evaluate the relative activities of the isolated rotenoids from *T. candida* along with a synthetic derivative, rotenonone (IV), taking pure rotenone (I) as the standard insecticide.

EXPERIMENTAL PROCEDURES

Instruments and Conditions. Melting points were determined on an electrically heated melting point apparatus and are uncorrected. UV spectra were recorded in EtOH on a Beckman

Table I. UV, IR, MS, and PMR Spectral Data of Amorpholone (III) Obtained from *T. candida*

UV	$\lambda_{\text{max}}^{\text{EtOH}}$ 307 nm
IR	$\gamma_{\text{max}}^{\text{KBr}}$ 3280 (OH), 2980, 1635 (C=O), 1590, 1510, 1450-1000, 900-700 cm^{-1}
MS	m/z 408 (M^+), 391, 379 (100), 363, 361
PMR	(DMSO- d_6) δ 8.54 (s, H-1), 8.08 (d, H-10, $J = 10 \text{ Hz}$), 7.84 (d, OH-6, $J = 7 \text{ Hz}$, exchanged), 7.08 (d, H-11, $J = 9 \text{ Hz}$), 6.74 (s, H-4), 6.2 (d, H-6, $J = 8 \text{ Hz}$), 5.56 (t, H-5', $J = 8 \text{ Hz}$), 5.08 (d, 2 H, H-7', $J = 10 \text{ Hz}$), 3.78 (s, 6 H, OMe), 3.60 (m, 2 H, H-4'), 1.78 (s, 3 H, 8'-Me)

Table II. Percentage Mortality of Fourth-Instar Larvae of *S. litura* (F.) by Different Extracts of *T. candida* and Rotenoids

sample	% mortality ^a at different doses ($\mu\text{g/g}$ of body wt) after 120 h			
	0.8	0.4	0.2	0.08
crude extracts				
petroleum ether	70	50	30	20
different fractions of MeOH extract				
benzene	50	30	10	
ethyl acetate	60	50	40	25
butanol	60	40	40	15
pure compounds				
rotenone	85	70	62	30
dehydrorotenone				
amorpholone	75	50	40	22
rotenonone	55	37	20	10

^a Average of three replicates.

DU-6 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer using KBr pellets. Mass spectral (EI-MS, direct insertion) studies were carried out on a JEOL JMS-DX 300 mass spectrometer at 70 eV. ¹H NMR spectra were recorded on JEOL JNM-Fx 100 spectrometer (100 MHz) using TMS as the internal standard.

Extraction. The air-dried stems and leaves (2.5 kg) of *T. candida*, collected from M/s United Chemical and Allied Products, Calcutta 1 (where a voucher specimen has been preserved), were cut into pieces and powdered with a mechanical grinder. The powdered plant material was extracted with petroleum ether (60-80 °C) in a Soxhlet apparatus for 30 h followed by methanol

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Table III. Results of Probit Analysis of the Mortality Data of *S. litura* Larvae

sample	regression eq	LD ₅₀ , µg/g of body wt	standard error	fiducial limits to the LD ₅₀ value, µg/g
crude extracts				
petroleum ether	$Y = 1.39X + 2.80$	0.38	0.11	0.23-0.63
different fractions of MeOH extract				
benzene	$Y = 2.09X + 1.06$	0.76	0.12	0.44-1.32
ethyl acetate	$Y = 0.88X + 3.58$	0.41	0.18	0.18-0.93
butanol	$Y = 1.11X + 3.10$	0.50	0.16	0.24-1.05
pure compounds				
rotenone	$Y = 1.48X + 3.23$	0.16	0.12	0.09-0.27
amorpholone	$Y = 1.37X + 2.95$	0.31	0.11	0.19-0.51
rotenonone	$Y = 1.44X + 2.36$	0.68	0.15	0.35-1.33

extraction for another 15 h. The concentrated MeOH extract was then dissolved in water and subjected to liquid-liquid partitioning successively with benzene, ethyl acetate, and butanol. Different doses of the petroleum ether extract and the C₆H₆, EtOAc, and BuOH extracted fractions of MeOH extract were then subjected to insecticidal tests.

Isolation of Dehydrorotenone (II) and Amorpholone (III). The petroleum ether extract of *T. candida* was placed on a silica gel chromatographic column (5 cm × 100 cm) and eluted with solvents and mixtures of solvents of increasing polarity (petroleum ether, C₆H₆, EtOAc, MeOH). From the benzene eluates dehydrorotenone (II) was isolated and identified by direct comparison with an authentic sample (Roy et al., 1986). The C₆H₆/EtOAc (9:1) eluates of the column provided light yellow crystals of amorpholone (III), crystallized from EtOH (mp 263 °C). III was then subjected to UV, IR, MS, and PMR spectral analyses for its characterization (Table I).

Preparation of Amorpholone (III) and Rotenonone (IV). III was prepared from 1.0 g of rotenone (I) (Sigma Chemical Co.) in a 10% overall yield according to the method described by Piatak et al. (1975) using N-bromosuccinimide as the brominating agent. The synthetic product was identical in all respects with the natural compound III.

Rotenonone (IV) was prepared from 50 mg of amorpholone (III) in 50% yield according to the method described by Piatak et al. (1975) using sodium dichromate. The product was identified by its melting point (295-298 °C) and IR spectrum [1740 (aromatic lactone system), 1630, and 1610 cm⁻¹]. The preparation of IV served as an alternative proof for the structural confirmation of III, and at the same time, its preparation provided the opportunity to evaluate the activity of the oxidation product of III.

Bioassay. The polyphagous insect, *S. litura* F., larvae were raised on *Corchorus capsularis* leaves at 24 (min) to 34 °C (max) and 75-85% relative humidity. Freshly molted fourth-instar larvae of average body weight 4.95 g were selected from the stock culture, starved for 12 h, and used individually for the assay of insecticidal activity. Appropriate solutions (in EtOH) of each of the crude extracts and pure compounds were prepared so that topical application of 4 µL of each solution incorporated 0.8, 0.4, 0.2, and 0.08 µg of the actual product per gram of body weight of each test larva. Sixty individuals of the test larvae were treated separately with each dose of each of the extracts and compounds tested with one set of individuals serving as untreated control. All of the larvae were provided with adequate food to avoid starvation. The percentage mortality was noted at different time intervals, and the final result (after 120 h) was subjected to Probit analysis (Finney, 1971) for computation of regression equations, LD₅₀ values (in micrograms per gram of body weight) and fiducial limits to the LD₅₀ values with 95% fiducial probability.

RESULTS AND DISCUSSION

Identification of Amorpholone (III). Column chromatography of the petroleum ether extract resulted in the isolation of dehydrorotenone (II) from the benzene eluates, and further elution of the same column with C₆H₆/EtOAc (9:1) provided yet another rotenoid, amorpholone (III), crystallized from EtOH (mp 263 °C) and analyzed for C₂₃H₂₀O₇ (M⁺ m/z 408). The spectral data of III are shown in Table I. Its IR spectrum showed an OH

absorption at 3280 cm⁻¹, aromaticity at 2980, 1590, 1510, 900-700 cm⁻¹, and a highly conjugated ketone at 1635 cm⁻¹ like rotenone. The identification of III as a rotenoid was further strengthened by its UV absorption maximum at 307 nm. The PMR spectral data of this compound (Table I) were in excellent agreement with those reported for amorpholone, previously isolated from a legume plant, *Amorpha canescens* (Piatak et al., 1975). The mass spectrum of III was also similar to that reported (Piatak et al., 1975) for amorpholone except the appearance of the base peak at m/z 379 (M⁺ - CHO) instead of at m/z 391 (M⁺ - OH). The genesis of the base peak at m/z 391 was explained by the earlier workers as due to the formation of a stable pyrylium ion from the ring to which OH was attached. However, in our case, the hemiacetal system of which the OH group is a part opens up to the aldehyde form which then loses CHO to give the base peak at m/z 379 (M⁺ - CHO). On the basis of the above spectral evidence, compound III has been deduced to be 6-hydroxy-6(a),12(a)-dehydrorotenone or amorpholone, which was further substantiated by its oxidation to a known product, rotenonone (IV), and confirmed by its partial synthesis from rotenone (I) (Piatak et al., 1975). Interestingly, the occurrence of III in the genus *Tephrosia* is being reported for the first time.

Insecticidal Activity of the Extracts of *T. candida* and Rotenoids. Slow action of rotenone as a stomach or contact poison is known (Fukami and Nakajima, 1971). So for the present investigation, the mortality of *S. litura* larvae after 120 h of application of the test products has been taken into consideration. The results reveal that mortality of 50% or more could be observed in the treatments of all of the extracts and most of the pure compounds at 0.8 µg/g of body weight of the larvae. Among the crude extracts the highest mortality of 70% was recorded for petroleum ether extract and among pure compounds it was 85% for rotenone (I). At the dose of 0.4 µg/g of body weight the petroleum ether extract and the benzene fraction of MeOH extract gave 50% mortality, and among pure compounds rotenone (I) and amorpholone (III) gave 70% and 50% mortality, respectively. Rotenone, even at 0.2 µg/g of body weight, gave as high as 62% mortality of the larvae. Dehydrorotenone (II) did not give any mortality even at the highest dose tested (Table II). Rotenone (I) was found to be superior to all of the pure compounds and extracts tested in the present investigation. All of the extracts were found to be promising at higher dose, and among these the petroleum ether extract proved to be the best. Development of suitable formulation and further tests on other groups of insects are required for adoption of at least the petroleum ether extract of *T. candida* as an alternative to synthetic insecticides in pest control.

Probit analysis on the data of mortality after 120 h of treatment revealed the toxicity in descending order as

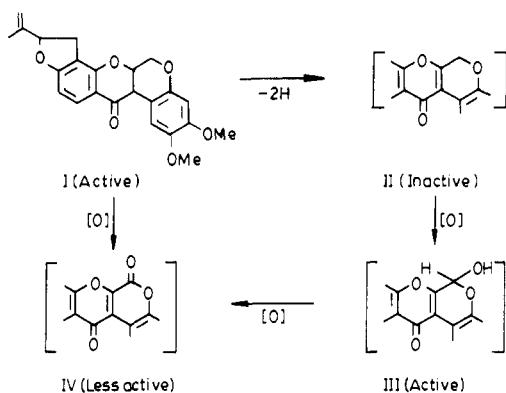


Figure 1. Structure-activity relationship among the rotenoids (I-IV): I, rotenone; II, dehydrorotenone; III, amorpholone; IV, rotenonone.

rotenone > amorpholone > petroleum ether extract > ethyl acetate fraction of MeOH extract > butanol fraction of MeOH extract > rotenonone > benzene fraction of MeOH extract. The relative toxicity of the test products stands as amorpholone 0.5, petroleum ether extract 0.4, ethyl acetate fraction of MeOH extract 0.4, butanol fraction of MeOH extract 0.3, rotenonone 0.2, and benzene fraction of MeOH extract 0.2, taking 1 for rotenone (Table III).

Structure-Activity Relationship. The varying degrees of insecticidal activity exhibited by the rotenoids (I-IV) may be explained on the basis of their structural features (Figure 1). The active compound I becomes completely inactive when dehydrogenated to II [6(a),12-(a)-dehydrorotenone]. But hydroxylation at the C-6 position converts inactive II to an insecticidally active form III, and when III is oxidized to IV, its chirality at C-6 is lost, thereby reducing the activity of III by 54%. Thus, it is quite evident from the present study that the C-6 position in the 6(a),12(a)-dehydrorotenone moiety (II) plays a significant role in conserving bioactivity, and one would need to select a suitable substituent for one of the H atoms at C-6 to produce an insecticidally active form of II.

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