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NOVEL SYNTHETIC ROTENOIDS WITH BLOCKED B/C RING SYSTEMS

Jonathan L. Josephs*, † and John E. Casida

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720

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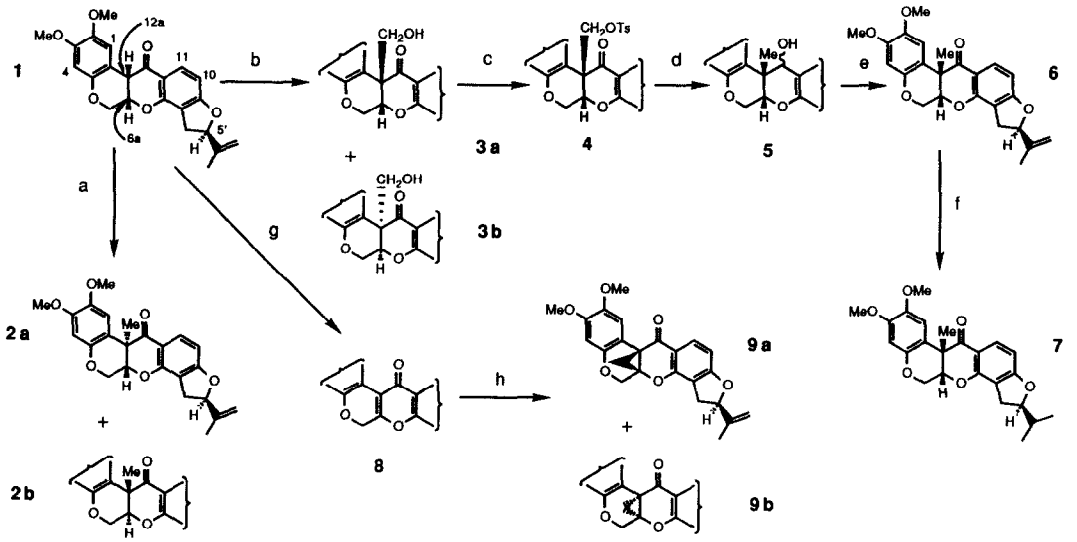
Abstract: The novel cis-12a-methylrotenone is much more potent than its trans epimer as an insecticide and inhibitor of NADH dehydrogenase, establishing the importance of the cis-B/C ring system for biological activity. Blocking the B/C ring fusion of rotenone with either the 12a-methyl or the 6a,12a-cyclopropyl substituent greatly increases the photostability and the 12a-methyl compound also has remarkably enhanced toxicity to houseflies.

Rotenone from *Lonchocarpus* or *Derris* has been used for centuries as a piscicide and for about 150 years as one of the two most important botanical insecticides.[1] It inhibits the NADH dehydrogenase segment of the respiratory chain and is effective on insect pests resistant to synthetic insecticides acting on other target sites.[2] Recent studies on the importance of the B/C ring configuration to NADH dehydrogenase inhibitory activity revealed that the unnatural *trans*-B/C ring system confers comparable activity to that of the natural *cis*-B/C ring system, a suprising observation on considering the pronouced change in molecular geometry unless the unstable *trans*-fusion epimerises to the stable *cis*-fusion under the assay conditions.[3,4] Rotenone's facile cytochrome P_{450}-mediated metabolism and photochemical oxidation are advantages in conferring short residual action but also limit its effectiveness. The 12a-position is one of the primary sites of metabolic and photochemical inactivation.[5,6] Novel synthetic rotenoids reported here with blocked B/C ring systems, either *cis* or *trans*, and with a 12a-methyl or a 6a,12a-cyclopropyl substituent allow two hypotheses to be tested: the *cis*-B/C ring configuration is preferred for activity; the labile 12a-position may be stabilised.

Methylation of rotenone (**1**) with methyl iodide and sodium hydride in DMF gave specifically *trans*-12a-methylrotenone[7] in 24% yield as the 6aS,12aR,5'R (**2a**) and 6aR,12aS,5'R (**2b**) diastereomeric pair which proved to be inseparable by HPLC;[8] there was no 6aS,12aS,5'R or 6aR,12aR,5'R *cis*-12a-methylrotenone, as the enolate is quenched to yield the kinetic *trans*-configuration and epimerisation to the thermodynamic *cis*-configuration is blocked. As an alternative route to 6aS,12aS,5'R-*cis*-12a-methylrotenone (**6**), rotenone was treated with formaldeyde in DMF to give, as reported,[9] the pair of diastereomeric alcohols 6aS,12aR,5'R (**3a**) and 6aR,12aS,5'R (**3b**) as a 50:50 mixture which was separated on silica with hexane/ethyl acetate. Spectral data verified the *cis*-stereochemistry.[9] The tosylate of **3a** was prepared and treated with two equivalents of LAH in THF to obtain the alcohol (**5**) which was smoothly oxidised to the desired **6** with manganese dioxide.[10] Hydrogenation using platinum oxide as catalyst in ethyl acetate gave *cis*-12a-methyldihydrorotenone (**7**).

†Present address; ISK Mountain View Research Center Inc., 1195 W. Fremont Avenue, Sunnyvale, CA 94087, USA



a: (24% yield) NaH, MeI, DMF, Reflux; **b**: (24% yield) HCHO, DMF, Reflux; **c**: (88% yield) TsCl, Pyridine, 0°C; **d**: (96% yield) LAH, THF, 0°C; **e**: (64% yield) MnO_2, CH_2Cl_2, RT; **f**: (80% yield) H_2, PtO_2, EtOAc; **g**: (24% yield) MnO_2, Me_2CO, Reflux; **h**: (75% yield) Me_3SOCl, NaH, THF, Reflux.

The ylide from trimethyloxosulphonium chloride[11] and sodium hydride in THF added solely in a 1,4-manner to 6a,12a-dehydrorotenone (**8**)[12] to yield 6a,12a-cyclopropylrotenone as a diastereomeric mixture of 6aS,12aS,5'R (**9a**) and 6aR,12aR,5'R (**9b**),[13] which proved inseparable by HPLC.[8]

The novel rotenoids were evaluated for potency as NADH dehydrogenase inhibitors[14] and as insecticides with or without the cytochrome P_{450} oxidase inhibitor piperonyl butoxide (PB) as a synergist[15] (Table). Rotenone (**1**) was 5-times more potent than *cis*-12a-methylrotenone (**6**) in the enzyme assay; since both compounds have similar conformations, this difference is presumably due to a steric effect.

Table. Potency of Rotenone and its Derivatives as Inhibitors of NADH Dehydrogenase and as Insecticides

rotenoid	NADH dehydrogenase IC_{50}, nM	Housefly LD_{50}, µg/fly -PB	Housefly LD_{50}, µg/fly +PB
1	3.5	10	5
6	17	2	0.2
2a + 2b	360	>10	4
7	16	10	1
9a + 9b	80	-	10

cis-12a-Methylrotenone (**6**) was 20-fold more effective than the mixture of *trans*-12a-methylrotenoids (**2a** + **2b**); clearly *cis*-ring fusion confers higher activity than *trans* and the reported activity of the labile *trans*-rotenone may be due to epimerisation to the more stable *cis*-geometry.[3,4] The activity of the cyclopropylrotenoids (**9a** + **9b**) is between that of the *cis*-(**6**)- and *trans*-(**2a** + **2b**)- 12a-methylrotenoids which is consistent with the B/C ring conformation of **9a** + **9b** being intermediate between the bent roof-tile like of the *cis* and the planar of the *trans*.[16] These data establish that the bent conformation of the *cis*-ring fusion is preferred for fit at the enyme active site. *cis*-12a-Methylrotenone (**6**) showed a 5-fold increase in insecticidal activity relative to rotenone (**1**) which was increased to 25-fold when tested with PB. *cis*-12a-Methyldihydrorotenone (**7**) also showed an increase in activity relative to **1**. The lower toxicity to houseflies of **7** relative to **6** is suprising in view of their comparable potency *in vitro*. **7** would be expected to be more stable than **6** therefore differences in transport may be involved. These improvements in toxicity to houseflies[17] are presumably due to blocking oxidative metabolism at the labile 12a-position, but improved penetration may also be a factor.

The 12a-blocked rotenoids should be more stable to photooxidation since with rotenone this process is initiated by cleavage of the C12a-H bond.[6] Photochemical decomposition in solution with HPLC for analysis[8] showed improved persistence relative to **1** with **6** being most stable. As thin films **6** had slightly enhanced stability relative to **1** (Figure).[18]

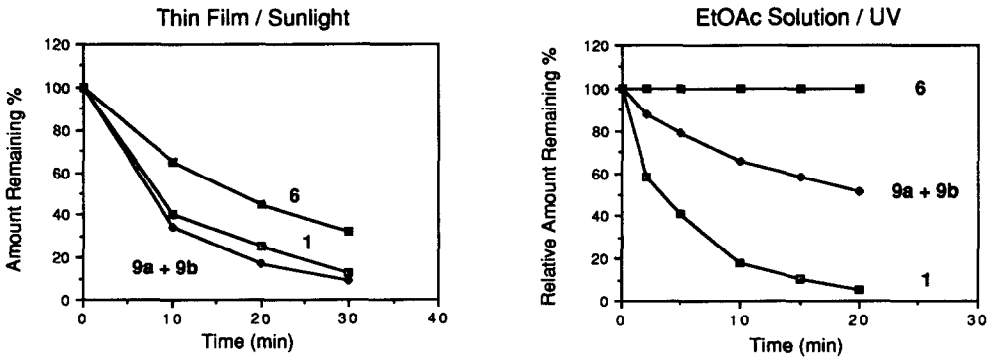


Figure. Relative stability of rotenone (**1**), *cis*-12a-methylrotenone (**6**) and 6a,12a-cyclopropylrotenone (**9a** + **9b**) as thin films (0.4 μg/cm^2) exposed to sunlight and as solutions in ethyl acetate (0.3 mg/ml) irradiated at 340 nm.

In conclusion, blocking the B/C ring junction of rotenone by replacing the 12a-proton with a methyl group or forming the 6a,12a-cyclopropyl derivative shows that the bent *cis*-fusion provides the best fit at the enzyme active site and that photochemical and metabolic reactions of the 12a-position limit the insecticidal activity and photochemical stability.

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8. Lichrosphere Si-60 analytical column, hexane/ethyl acetate.
9. Kostova, I. N.; Ognyanov, I. V. *Z. Naturforsch.* **1983**, *38b*, 761. The absolute stereochemistry is correctly identified but the R,S nomenclature is incorrectly assigned since introducing a hydroxymethyl group at C-12a changes the priority order of the substituents.
10. **6** ^{1}H NMR: (300 MHz, $CDCl_3$) ∂ 1.60 (3H, s, 12a-Me), 1.76 (3H, s, 8'-Me), 2.94 (1H, dd, *J* 15.8 and 8.2 Hz, 4'-H), 3.30 (1H, dd, *J* 15.8 and 9.8 Hz, 4'-H), 3.75 (3H, s, OMe), 3.80 (3H, s, OMe), 4.38 (1H, d, *J* 11.3 Hz, 6-Ha), 4.56 (1H, dd, *J* 11.5 and 2.9 Hz, 6-Hb), 4.58 (1H, bs, 6a-H), 4.93 (1H, s, 7'-H), 5.07 (1H, s, 7'-H), 5.23 (1H, dd, *J* 9.8 and 8.2 Hz, 5'-H), 6.43 (1H, s, 4-H), 6.50 (1H, d, *J* 8.6 Hz, 10-H), 6.70 (1H, s, 1-H), and 7.83 (1H, d, *J* 8.6 Hz, 11-H).
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13. **9a** + **9b** ^{1}H NMR: (300 MHz, $CDCl_3$) ∂ 1.77 and 1.79 (3H, s, 8'-Me), 2.02 (2H, s, cyclopropyl), 2.98 and 3.01 (1H, m, 4'-H), 3.31 and 3.36 (1H, m, 4'-H), 3.82 (3H, s, OMe), 3.95(3H, s, OMe), 4.30 (1H, d, *J* 10.9 Hz, 6-Ha), 4.52 (1H, d, *J* 10.9 Hz, 6-Hb), 4.95 (1H, bs, 7'-H), 5.09 (1H, bs, 7'-H), 5.29 and 5.33 (1H, m, 5'-H), 6.46 (1H, s, 4-H), 6.59 (1H, d, *J* 8.6 Hz, 10-H), 7.87 (1H, d, *J* 8.6 Hz, 11-H), 8.36 and 8.37 (1H, s, 1-H).
14. NADH dehydrogenase activity was measured spectrophotometrically in 0.25 M sucrose/50 mM potassium phosphate, pH 7.6 with 0.28 mM NADH. Singer, T. P. *Methods of Biochemical Analysis*; Glick, D., Ed.; John Wiley: New York, 1974; Vol 22, p 123
15. Adult female houseflies (*Musca domestica* L., SCR strain) treated individually and topically with PB (5 μg) in 0.5 μl acetone followed 1hr later by the rotenoid in 0.5 μl acetone, with mortality determinations at 24 hr.
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17. Introducing the *cis*-12a-methyl substituent reduces the toxicity to male mice treated intraperitoneally (24 hr LD_{50} values of 2.8 and 5 mg/kg for **1** and **6** respectively) thereby improving the selective toxicity in poisoning houseflies relative to mice. In comparisons with other insects, **1** is more toxic than **6** to *Apis mellifera* and *Oncopeltus fasciatus* on topical application and by 30-fold to *Trichoplusia ni* third instar larvae on ingestion of treated bean leaves.
18. Photodecomposition experiments were carried out with a mixture of the three compounds for internal comparison. *cis*-12a-Methylrotenone (**6**) was used as the internal standard for the solution study (<10% decomposition during irradiation).