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THE ROTENOID CORE STRUCTURE: MODIFICATIONS TO DEFINE THE REQUIREMENTS OF THE TOXOPHORE.

Leslie Crombie,*[a] Jonathan L. Josephs,[†] [a] Jane Cayley,[b] John Larkin[b] and John B. Weston.[b]

[a] Department of Chemistry, The University of Nottingham, Nottingham, NG7 2RD, UK.
[b]Wellcome Research Laboratories, Ravens Lane, Berkhamsted, Hertfordshire, HP4 2DY, UK.

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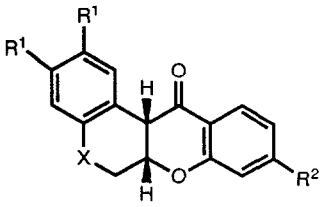
Abstract: A number of modified analogues of the core structure **1** , representing the simplest rotenoid, have been made and assayed for their inhibition of NADH dehydrogenase in a blow fly flight-muscle submitochondrial preparation.

In many tropical countries in the world rotenoid bearing plants have been used by native fishermen as fish poisons to stupefy fish prior to capture, e.g. the use of *Lonchocarpus* species by South American Indians. Well before the constituents were known, ground root preparations from *Derris elliptica* were used as garden insecticides in the East Indies, [1] and are still commercially available today as 'Derris Powder'. Its status as a botanical insecticide [2] and its high potency for control of some pests which have become resistant to some pyrethroids [3] has led to continued interest. The main bioactive principle is rotenone **7 b**, which exhibits its activity through inhibition of the NADH dehydrogenase segment of the respiratory chain [4,5] and has long been used as a biochemical probe for this system.[6] Fukami's study [7] of a series of rotenoid derivatives was the first attempt to relate chemical structure to toxicity and two sets of work on the inhibition of mitochondrial respiration have been carried out with limited sets of rotenoids. [8,9] Certain features needed for effective inhibition could be determined but these gave little insight into the nature of the inhibition site. The shortfalls of these studies appear to be due to the set of compounds being defined on the basis of availability from extraction or unrelated synthetic studies, rather than a planned synthetic effort. We hope to define the rotenoid toxophore by synthesising and testing a systematic set of analogues which have specific modifications based on the rotenoid core structure **1**. Munduserone **4** is the simplest naturally occurring rotenoid, and this oxygenation pattern is a common theme among the rotenoids. [10] We therefore wished to investigate the role of these methoxy groups on activity by testing a set of methoxy substituted core structures **1** - **4**. The heteroatoms in the B- and C-rings may play an important role in binding and we therefore felt that substituting sulphur for oxygen in the B-ring would provide information about the electronic requirements at this position. In addition the increased carbon - heteroatom bond lengths might effect conformational changes; thus compounds **5** and **6** were required. The requirements of the B/C-ring junction stereochemistry need to be elucidated and our recently discovered method for the synthesis of unnatural *trans*-B/C rotenoids [11] provides us with compounds **7** - **10** for comparison.

The required compounds were synthesised by literature methods for those compounds which had

[†] Present address: Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, CA 94720, USA.



1 $R^1 = H$ $R^2 = H$ X = O
2 R^1 = MeO $R^2 = H$ X = O
3 $R^1 = H$ R^2 = MeO X = O
4 R^1 = MeO R^2 = MeO X = O
5 $R^1 = H$ $R^2 = H$ X = S
6 R^1 = MeO $R^2 = H$ X = S

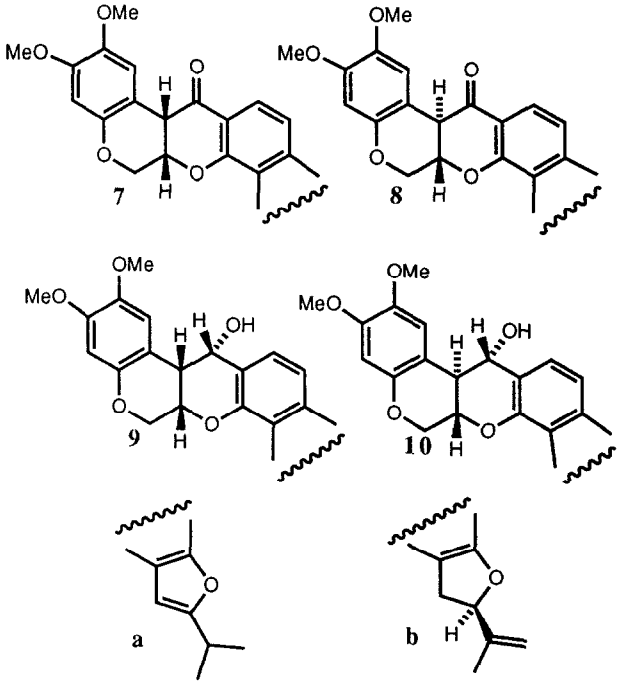


Table. INHIBITION OF NADH DEHYDROGENASE. [23]

COMPOUND	INHIBITION IC_{50}(M)	RELATIVE ACTIVITY % (to rotenone)
1	5.0×10^{-6}	5.0×10^{-3}
2	2.0×10^{-7}	1.25×10^{-1}
3	8.0×10^{-6}	3.1×10^{-3}
4	1.5×10^{-8}	1.67
5	1.0×10^{-5}	2.5×10^{-3}
6	3.0×10^{-7}	8.3×10^{-2}
7b	2.5×10^{-10}	100
8b	5.0×10^{-10}	50
9a	2.5×10^{-8}	1.00
10a	4.0×10^{-7}	6.3×10^{-2}
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7b	1.5×10^{-9}	100
7a	2.0×10^{-9}	75
8a	5.0×10^{-9}	30

previously been reported or could be readily made by existing methodologies. The remaining compounds were synthesised by routes we specifically developed to allow for the introduction of novel functionalities and these methods are reported elsewhere. [11,12] The core structure **1** was made by a modification [13] of LaForge's method.[14] Compounds **2** and **4** were synthesised by an acetylenic Claisen rearrangement route.[15] However formation of **3** was not successful by this route and was obtained as colourless needles m.p. 151 - 152°C by using the methodology of Verhé. [18] Compounds **5** and **6** are not obtainable by existing routes and new methodology was developed for this purpose, the key step being an intramolecular Mukaiyama reaction. This route provided both of the desired compounds. [12] Rotenone **7b** was available by extraction from 'Timbo resin', whilst isorotenone **7a** was obtained by isomerisation of natural rotenone **7b**. [19] The unnatural *trans*-rotenoids **8a, 8b** were synthesised by diisobutylaluminium hydride reduction of the corresponding 6a,12a-dehydrocompounds.[11] Alcohols **9a** and **10a** were obtained by sodium borohydride reduction of the corresponding ketones **7a** and **8a**. [20]

The compounds were assayed for their ability to block NADH dehydrogenase in a preparation of submitochondrial particles obtained from blow fly flight-muscle,[21] the conversion of NADH to NAD being monitored spectrophotometrically with time at 340 nm.[22] Results are expressed as IC_{50} and relative (to rotenone) % activity values.[23]

A comparison of structures **1** - **4** permits one to see the effect of the methoxy groups on the A- and D-rings. Comparison of **1** and **3** shows that in isolation the methoxy group on the D-ring has little effect on activity. However, comparison of **2** and **4** shows that in the presence of methoxy groups on ring-A, the D-ring substituent causes an order of magnitude increase in activity. Consideration of **1** and **2** indicates that in isolation the methoxy groups on ring-A cause an order of magnitude increase in activity, whilst juxtaposition of **3** and **4** shows a two orders of magnitude increase in activity (due to ring-A methoxylation) in the presence of a methoxy group on ring-D. Three methoxy groups on rings -A and -D, as in munduserone, thus cause a 2 orders of magnitude increase in activity. Comparisons of **1** and **5**, and **2** and **6**, show that substitution of sulphur into ring-B in place of oxygen results in similar levels of activity. Again, in the sulphur series, the introduction of methoxy groups into ring-A causes an increase in activity.

The effect of the B/C-ring junction stereochemistry may be analysed by comparison of *cis*- and *trans*-rotenone **7b** and **8b**, and (±)-*cis*- and *trans*-isorotenone **7a** and **8a**, which indicates that there is no substantial loss of activity in going from the *cis*- to the *trans*- fusion despite the strong change in molecular geometry. This result seemed somewhat surprising and raised the possibility that the readily epimerisable *trans*-B/C fusion may be converted into the *cis*-B/C-fusion under the assay conditions. The 12a-α-hydroxy compounds **9a** and **10a** were therefore prepared, since they would not be enolisable and preserve the B/C-stereochemistry. They both have lower activity with the *trans*- alcohol **10a** showing distinctly greater loss of activity. In our opinion the role of the B/C-ring fusion geometry is not fully clarified and warrants studies of additional unenolisable *cis*- and *trans*- rotenoids. It is clear from this work however that the aromatic methoxylation makes a distinctive contribution to activity, whilst the replacement of oxygen by sulphur in ring-B has little effect despite the lengthened bonds and distortion in ring shape.

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22. The particle suspension (2µl) was added to the isolation buffer (100µl, 0.3M sucrose, 20mM potassium phosphate, 2mM EDTA, pH7.6) in a semimicro cuvette (1ml). The sample (1µl solution in acetone) was added and incubated at 30°C for 15 min. The sample was diluted with reaction buffer (880µl, 0.16M aspartate , 5mM potassium phosphate, 1mM EGTA, 0.02mM magnesium chloride, pH7.4). The assay was initiated by the addition of NADH (20µl, 10mM solution). Conversion of NADH to NAD was monitored at 340nm spectrophotometrically with time at 30°C.
23. Assays of compounds **7a** and **8a,** along with rotenone as standard, were performed on a separate occasion. Relative activity values (%), are with respect to rotenone.