Synthetic studies on electron transport inhibitors related to natural products

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Introduction

The natural O-heterocycle rotenone (1) is a classical inhibitor of electron transport, and an effective insecticide [1–3] and piscicide. Its current commercial uses are confined to horticulture (a 'green' pesticide) and fish farming. Structure–activity relationships have been investigated a number of times [4–6]. These studies have relied heavily on the natural rotenoids, and compounds derived from them by chemical transformation with a limited number of wholly synthetic samples. Not all the results of such studies are in the public domain, but the data known to the authors support two broad generalizations: (i) the natural rotenoids, with an intact A/R/C/D system and ring AID substitution, are active inhibitors; and (ii) few derivatives of the natural rotenoids (in which the B/C system is altered) are active, and cleavage of these rings results in major loss of activity. Thus, there remains a narrow range of active inhibitors, with a complex basic structure that is hard to synthesize. To make progress in this area, there appeared to us to be two main requirements. First, the development of a new synthesis of rotenoids, which should be short, practical, and give a good overall yield. Although a number of syntheses of the A/B/C/D ring system have been devised [7, 8] they have not been very suitable for the preparative needs of structure–activity investigations. A new approach to this problem is addressed in the first part of this paper. Secondly, new ideas are needed for active structural variants which will not have to contain the core rotenoid tetracycle. We decided to look for inspiration at structural comparisons between rotenone and other inhibitors that are believed to act at the same site (NADH:ubiquinone oxidoreductase) [9]. Our progress in this area is described in the second section.

Discussion

Synthesis of the rotenoid ring system

Previous syntheses have been dominated by the formation of the C ring under thermodynamic control, which provides the necessary cis ring junction. This tactic requires the presence of the 12-carbonyl function to stabilize the 12a enol/enolate. Since a few active compounds lack this ketone function, e.g. the alcohol (2), we aimed to build a synthesis with kinetic control of stereochemistry. We chose a strategy involving ring construction in the order D→C→A→B, with aryl to carbon bond formation as the final stage. After various explorations the route outlined in Scheme 1 emerged [10]. Convenient starting materials are the o-hydroxyacetophenones (3); many substituted variants are available. Claisen reaction with ethyl methylthioacetate under anhydrous basic conditions afforded the 2-methylthiomethylchromone (4). Refluxing the latter with methyl iodide gave the corresponding iodide (5), by S-methylation and iodide ion substitution on the sulphonium salt. Reaction with a range of phenols under weak base conditions provided the o-iodoaryloxymethylchromones (6), containing rings A, C, and D. The chromone was set up for cyclization by first reducing the chromone C–C

Received 27 July 1993

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double bond selectively with diisopinocamphenylborane to the chromanone, and then forming the enol acetate (7) with isopropenyl acetate and acid catalyst. Final ring closure was effected by treatment with tributyltin hydride in refluxing benzene, with a radical initiator. Abstraction of iodine using tin radicals led to the corresponding aryl radicals which cyclized under kinetic control, with axial approach to the enol double bond; the product radical abstracted hydrogen on the least hindered (β) face to afford the tetracycle (8, R = Ac), with the cis-cis stereochemistry of the (racemic) rotenoid alcohol (2). This route comprises five steps from the initial chromones, readily available in multigram quantities, with an overall yield (unoptimized) of 15%. The methods are tolerant over a range of functionality. We believe that this synthesis opens the way for convenient production of a new range of synthetic rotenoids. It was hoped that the reduction of chromone (6) with the chiral reagent diisopinocamphenylborane would be enantioselective, but this proved not to be the case. At present we are examining enzymic hydrolysis of acetate (8), a promising means of accessing homochiral products.

**Synthesis of hybrid inhibitors**

We have undertaken a number of simple molecular modelling and structure comparison studies between rotenone and other inhibitors, e.g. piericidin A; myxalamide D; benzimidazole derivatives; papaverine, etc. Based on these observations, we have explored the synthesis of a number of 'hybrid' organic molecules with appropriate fragments of different inhibitors. In this paper two sets are discussed. The first group are represented by formula 9 (Scheme 2); rotenone rings A/B have been replaced by 2-methylbenzimidazole and ring C is

![Scheme 1](image-url)
deleted. Various combinations of oxygen function are included. It was envisaged that such compounds could be prepared from 2-methylbenzimidazole (10) and the corresponding chloride (11). The acid chloride (11; X = O, Y = OMe) was obtained from the known tubaic acid [2] derived through degradation of rotenone. The second acid chloride was synthesized (Scheme 2) from phenol using the Nickl reaction shown, providing the dihydrobenzofuran (12); formylation to 13, oxidation and chlorination followed in straightforward fashion to yield the required product (14). Reaction with 10 followed the desired course. The benzyl alcohol (16) was made from dimethyl tubaic acid (15), via ester hydrolysis, activation of the carboxylic acid (to allow low-temperature reduction) and treatment with sodium borohydride at -60°. Aldehyde 14 was reduced to 17. It was found to be most convenient to react the alcohols 16 and 17 directly with 10, using the Mitsunobu procedure with diethylazodicarboxylate, rather than proceeding through the corresponding benzyl chlorides.

The second hybrid inhibitor for discussion here is the prenylisooquinoline derivative (Scheme 3, 18; R = H/OMe) with rotenoid rings A, D, and E, but B/C replaced by an N-heterocyclic unit as suggested by overlay with tetrahydropapaverine. The planned synthesis involved an intermediate amide (19), derived from the amine (20). Scheme 3 indicates the actual successful route. The aryl iodide
(21) was converted to the dihydrobenzofuran (22) by palladium-catalysed isoprenylation. The double bond was protected by addition of thiophenol, to form 23, which was taken on conventionally through 24 and 25 to the required amine (26). Coupling of this amine to 3,4-dimethoxyphenylacetic acid gave the amide (27). Ring closure by the Bischler–Napieralski method, followed by imine reduction yielded the tetrahydroisoquinoline (28). Finally, the double bond was revealed in a sequence involving N-trifluoroacetylation, oxidation of sulphide to sulphoxide; thermal elimination of phenyl sulphenic acid; and N-deacylation.

The six compounds (11, X = O, Y = OMe; 11, X = O, Y = H; 11, X = H₂, Y = OMe; 11, X = H, Y = H; 18, R = H; and 28) were then tested for inhibitory activity using a preparation of insect flight muscle. All six compounds proved to inhibit electron transport, with pIC₅₀ (i.e. −log₁₀ IC₅₀) in the range 7.90–6.60, cf. rotenone with pIC₅₀ 8.70. Although these activities are not outstanding, we believe it is of interest that the molecules are good inhibitors, representing as they do the rotenoid structure with deliberately redesigned B/C rings. Further work with other ‘hybrids’ is projected.

We are grateful to the S.E.R.C. and Wellcome Environmental Health for financial support, to Dr Jane Cayley (Wellcome) for the biological evaluations and to Dr John Weston (Wellcome) for valuable discussions.

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Mitochondrial Electron Transport Inhibitors


Received 27 July 1993

The thiourea insecticide diafenthiuron inhibits mitochondrial ATPase in vitro and in vivo by its carbodiimide product
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Introduction
Thioureas are a relatively new class of insecticides and acaricides of hitherto unknown mode of action. One of these, diafenthiuron, a product especially effective against sucking pests, has recently been introduced into the market [1]. So far, we have not found any direct target for diafenthiuron itself to explain its toxic effect. However, diafenthiuron is easily desulphated in vitro and in vivo to the pesticidal carbodiimide, CGA 140408. None of the known molecular targets of current commercial insecticides is affected by CGA 140408 (e.g. acetylcholinesterase, sodium channel, acetylcholine receptor, chitin synthesis, octopamine receptor). However, CGA 140408 is a potent inhibitor of mitochondrial respiration in vitro. It selectively blocks the coupling site and mitochondrial F0/F1-ATPase activity in a time- and concentration-dependent manner resembling dicyclohexylcarbodiimide (DCCD). In insect mitochondria, both carbodiimides bind covalently to the proteolipid subunit of the F0/F1-ATPase in the inner mitochondrial membrane and to porin, a channel-forming protein of the outer mitochondrial membrane. In vivo, CGA 140408 has a depressing effect on locomotive activity and on respiration like other mitochondrial inhibitors. In intoxicated locusts, ATPase activity is blocked up to 80% in vivo. A corresponding decrease of ATP concentration is also observed. The extent of inhibition of ATPase parallels the degree of intoxication and may differ in individual tissues. Tissues that are affected are the nervous system, jumping leg muscle and the gut.

Our data suggest that inhibition of energy metabolism in mitochondria is the primary mode of action of CGA 140408 in vivo.

Diafenthiuron as a pro-insecticide
There is strong evidence that diafenthiuron is only a pro-insecticide and that the carbodiimide, CGA 140408 [2], the highly active photoproduc of diafenthiuron, is responsible for its insecticidal activity: (a) the efficacy of field trials exceeds that in greenhouse experiments; (b) an excellent vapour phase activity is observed although diafenthiuron has only a low vapour pressure; (c) the pesticidal activity of diafenthiuron increases with time; (d) in vitro, diafenthiuron can be converted to CGA 140408 in a cytochrome P-450-dependent reaction; (e) diafenthiuron is converted to CGA 140408 by singlet oxygen which can be generated by sunlight [2]; (f) in vivo, CGA 140408 is a metabolite of diafenthiuron [3]; (g) the cytochrome P-450 inhibitor piperonyl butoxide (PBO) decreases toxicity of diafenthiuron (Figure 1) (which indicates an important contribution of P-450 to the metabolism of diafenthiuron to a toxic product, presumably CGA 140408); and (h) no target for diafenthiuron has yet been identified in vitro [3].

Possible targets
We applied test systems which included the targets of the major commercial insecticides [3]: CGA 140408, but not diafenthiuron, was a potent inhibitor of the coupling site in mitochondria. In our systems in vitro [3], CGA 140408 did not affect the octopamine receptor as suggested by Kadir and Knowles [4]. Moreover, we found no evidence that CGA 140408 would stimulate adenylate cyclase (Figure 2).