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THE UNCOUPLING ACTIVITY OF SUBSTITUTED ANTHRACENE DERIVATIVES ON ISOLATED MITOCHONDRIA FROM *PHASEOLUS AUREUS*

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Key Word Index—*Phaseolus aureus*; Fabaceae; mitochondria; anthracenes; anthraquinones; uncoupling activity.

Abstract—Unsubstituted anthraquinones and anthrones have a low uncoupling activity on isolated mitochondria. The presence of phenolic groups is a prerequisite for a marked effect. The number of phenolic groups is of minor importance but their substitution pattern is important. The methylene group of the anthrones increases the activity.

INTRODUCTION

In 1972, Van Sumere *et al.* [1] indicated that salicylaldehyde, β-resorcylaldehyde and naphthoquinones uncouple oxidative phosphorylation and increase mitochondrial respiration. Hemker [2], and Gladtke and Liss [3] concluded that the uncoupling activity of phenolics is determined by the amount dissolved in mitochondrial lipids. At a concentration of 10^{-3} M most quinones are inhibitors of oxygen consumption by mitochondria. The drug Dithranol (1,8-dihydroxyanthrone) appears to cause in different experiments a marked decrease in oxygen consumption of the mitochondria in the human epidermis [4, 5]. However, at lower concentrations quinones stimulate yeast respiration, showing an uncoupling instead of an inhibitory effect. It seems that stimulation or inhibition is mainly determined by a suitable combination of both structure and concentration. As pH is connected with dissociation of drugs, it is also a factor influencing the activity [6]. Kean [7] reported in 1970 biological activity associated with rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid or 1,8-dihydroxyanthraquinone-3-carboxylic acid). He found that its inhibition of electron transport showed a marked degree of specificity for NADH-linked oxidations. He also observed a complete lack of effect on coupled phosphorylations, concluding that complex I was involved in the effect. He found it unlikely [8] that effects on sulphydryl groups are involved in the inhibition since mercaptoethanol did not give protection. Another possibility, which can be excluded by experiments with artificial electron carriers, is that rhein participates in shunt pathway of electron transport.

Since anthrones have a specific 'active methylene' group as part of their structure, we have now tested their effect on coupled phosphorylation in relation to the corresponding oxidized forms, the anthraquinones.

RESULTS AND DISCUSSION

The correlation between mitochondrial uncoupling and the concentration of uncoupling agent enabled us to calculate the concentration causing 25% uncoupling. The results are summarized in Table 1.

It is obvious that the parent compounds, anthraquinone and anthrone, have a very low uncoupling activity. Thus, the presence of a phenolic group is a prerequisite for a marked activity. The presence of phenolic groups does not necessarily produce activity, and an increase in their number does not necessarily enhance activity. The 1,4- and 1,5-dihydroxy derivatives, indeed show no activity and the other dihydroxy derivatives are more active than the corresponding tri- or tetrahydroxy derivatives. In the series of dihydroxy compounds, the substitution pattern seems to be important. Free phenolic groups produce activity; however, this is not always necessary for activity, since two of the most active compounds, 1-hydroxyanthraquinone and related anthrone, have hydroxyl groups which are chelated with the adjacent carbonyl group.

By comparing the anthraquinones and the corresponding anthrones, it is clear that, with the exception of 1,8-dihydroxy-9-anthrone, all the anthrones are as active as or more active than the anthraquinones. Thus the methylene group is of some importance and may enhance activity. All anthrones tested are 9-anthrones unless otherwise stated. Protection of the methylene group by the adjacent hydroxyl groups as in 1,8-dihydroxy-10-anthrone (**1**) causes a marked decrease in uncoupling activity compared with the corresponding 9-anthrone (**2**).

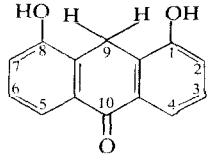
A special case is 1,8-dihydroxyanthrone-3-carboxylic acid; while the anthraquinone (rhein) is completely devoid of activity, the anthrone is a very efficient uncoupler. It is already known that rhein has no uncoupling properties, but is an inhibitor of elec-

Table 1. Comparison of anthracene derivatives

Compounds	Concentration of injected solution (mg/100 ml MeOH)	Final concentration obtained (μM)	Concentration causing 25% uncoupling (μM)*
Anthraquinone	33.6	34.3	—
Anthrone	248.2	272.4	47.0
Anthracenetriol	1016.8	299.7	55.0
2-Aminoanthraquinone	628.1	234.4	—
1-Hydroxyanthraquinone	59.5	22.3	5.0
1-Hydroxyanthrone	43.0	10.9	4.0
1,2-Dihydroxy-anthraquinone	143.2	95.5	40.0
1,2-Dihydroxyanthrone	20.0	4.7	2.0
1,4-Dihydroxy-anthraquinone	6.2	5.4	—
1,4-Dihydroxyanthrone	42.0	29.6	—
1,5-Dihydroxy-anthraquinone	3.3	1.8	—
1,8-Dihydroxy-anthraquinone	30.3	14.7	5.0
1,8-Dihydroxy-9-anthrone	41.0	24.2	10.0
1,8-Dihydroxy-10-anthrone	97.6	45.6	24.0
1,8-Dihydroxy-anthraquinone-3-carboxylic acid	11.9	7.0	—
1,8-Dihydroxy-anthrone-3-carboxylic acid	95.2	37.5	7.0
2,6-Dihydroxy-anthraquinone	84.2	29.2	13.0
2,6-Dihydroxyanthrone	24.0	14.1	4.0
1,2,4-Trihydroxy-anthraquinone	79.7	41.5	11.0
1,2,5,8-Tetrahydroxy-anthraquinone	33.7	23.1	18.0
2,2-Dihydroxy-benzophenone	402.1×10^3	21.2×10^3	206
4,4-Dihydroxy-benzophenone	35.1×10^3	4.3×10^3	248
Bis (4-hydroxyphenyl)-methane	267.0×10^3	39.0×10^3	3210

*A dash indicates that no marked activity could be detected.

(**1**) 1,8-dihydroxy-10-anthrone
(**2**) 1,8-dihydroxy-9-anthrone
(**3**) 2,2-dihydroxybenzophenone
(**4**) 4,4-dihydroxybenzophenone
(**5**) Bis (4-hydroxyphenyl) methane



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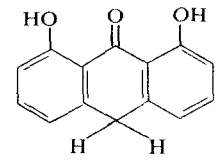

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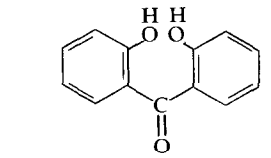
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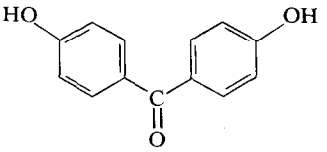
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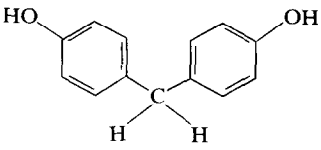
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tron transport [7, 8] and our results are in good agreement. Probably the fact that the carboxyl group is partly ionized at pH 7.2 may influence the electronic properties of the molecule, thus creating transport problems. The methylene group here seems to be very important.

The presence of a planar structure in these anthracenes is perhaps another important factor determining activity (see Table 1). The three non-planar structures tested (**3–5**) exert only a low uncoupling activity. High concentrations of these substances cause intense inhibition of respiration. If it were possible to use anthracene derivatives at the same concentrations, they would probably show the same activity pattern.

In summary, the requirements for uncoupling activity in the anthracene series include: (1) a rigid, planar structure; (2) presence of up to two free hydroxyl groups, preferably in positions 2, 3, 6 or 7; and (3) the presence of a methylene group in the *meso*-position, without adjacent hydroxyls.

EXPERIMENTAL

Preparation of mitochondria. Mitochondria were isolated from dark-grown, 4–5-day-old mung bean hypocotyls. The isolation procedure and the composition of the media was as described by Ikuma and Bonner [9].Finally the mitochondrial pellet, isolated from 40 g beans was taken up in 1 ml washing medium. For each experiment we used 2.9 ml of a medium containing mannitol, $MgCl_2$, KCl, phosphate buffer and bovine serum albumin (BSA) and 100 μl mitochondrial suspension in the respiration cell. The method of Lowry [10] was used to calculate the mitochondrial protein content with BSA (Sigma) as standard. All chemicals used were of analytical grade and solns were prepared in bidistilled H_2O.

Assay of uncoupling. All the compounds tested were dissolved in MeOH as the solvent in its final concn did not affect mitochondrial activity. Solns of anthracene derivatives were kept at room temp., but the unstable anthrone was kept in soln at 0°. The concn of the satd anthraquinone solns was determined spectrophoptometrically and the concn of the anthrone solns by the method of ref. [11]. Small quantities (2–8 μl) of the freshly prepared saturated test soln were added to the stirred mitochondrial suspension. Exactly 1 min after injection of the test soln, a small quantity of ADP (2 μl 50.0 mM) was injected. The oxygen consumption was followed in an Oxygen Clark Monitor (Yellow Springs Co., Cleveland).

The RC and the ADP/O were determined. In one single run, from 100 to 0% oxygen saturation, 5–7 injections were made. As both the RC and the ADP/O index are criteria for the intactness of mitochondria, rapid changes in both indexes were expected. The respiration rate increased as a function of the degree of uncoupling. The state of total uncoupling (100%) is that state in which an additional dose of ADP showed no more change in respiration rate and gave a RC of 1.0 and an ADP/O of 0, and an appropriate injection of dinitrophenol (DNP) caused no increase in respiration. It was assumed that the initial value found for RC, without any uncoupling agent, showed 0% uncoupling. As the first injection of ADP always showed a slower response, two injections were made and the results of the second used for the calculation. The degree of uncoupling was measured by means of Δ% RC and ADP/O. Although the results obtained by this method are probably difficult to compare with those obtained by other methods, it allows a good comparison between the tested compounds. We found a good correlation between dose and activity, which allowed us to calculate the 25% uncoupling dose. Each experiment was repeated two or more times to establish consistency. Thus the data presented are the means of at least two sets of concordant results.

Material. 1,8-Dihydroxyanthraquinone-3-carboxylic acid was prepared from aloin by the method of ref. [12]. 1,8-Dihydroxyanthrone-3-carboxylic acid and 1,8-dihydroxyanthrone were prepared by reduction of the corresponding acetylated anthraquinone with $SnCl_2$ in acid medium, according to ref. [13]. 2,6-Dihydroxyanthrone was prepared by reduction of 2,6-dihydroxyanthraquinone in aq. Na_2CO_3, with sodium dithionite. Other anthrones were prepared by reduction of the corresponding anthraquinones with $SnCl_2$ in acid medium, according to ref. [14]. All products were tested for purity by TLC.

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