light cycling (*16*). In noting the efficacy of pentobarbital as a circadian zeitgeber, we cannot overlook the fact that low levels of phenobarbital in the diet are cocarcinogenic (*25*) or the possibility that the act of resetting a circadian oscillation by means of chronobiotic drugs or otherwise inevitably implies molecular-genetic manipulation (*26*) with attendant mutagenic and carcinogenic sequelae (*27*).

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27. Work in progress has shown that rats fed phenobarbital in the diet at dose levels that are cocarcinogenic have exhibited patterns of temperature control that are more dysphasic than circadian [see C. F. Ehret, V. R. Potter, A. McQueen, *Argonne National Laboratory Report 8070* (1973), p. 160]. Evidence relating to the deleterious action of aperiodic environments and loss of internal temporal organization has been cogently reviewed by C. S. Pittendrigh and D. H. Minis [*Proc. Natl. Acad. Sci. U.S.A.* **69**, 1537 (1972)].
28. This research was conducted in large part at the University of Wisconsin, Madison, Biotron, a controlled-environment research facility supported by the National Science Foundation and the University of Wisconsin. We thank the Biotron staff, especially L. Anderson and W. Handel for assistance in instrument maintenance and animal care at Madison; W. Eisler and J. Blomquist for help in data acquisition, programming, and processing at Argonne; M. Lichtenstein, P. Grothus, H. Burke, N. Linden, and J. Robinson for their help in animal and data handling; and Drs. C. Peraino and A. Winfree for helpful suggestions. The work was supported in part by the AEC and by the Research Committee of the Graduate School, University of Wisconsin, Madison.

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Flavonoids as Inhibitors of Lens Aldose Reductase

Abstract. *Flavonoids are effective inhibitors of lens aldose reductase. Quercetin, quercitrin, and myricitrin are significantly more potent than the previously known aldose reductase inhibitors. The inhibitory activity is of the noncompetitive type. In addition, quercitrin effectively blocks polyol accumulation in intact rat lenses incubated in medium containing high concentration of sugars.*

Although the ubiquitous distribution of flavonoids in the plant kingdom has been known for a long time, a biological action of this group of compounds in animals and man was first suggested by Szent-Györgi and his colleagues (*1*) who reported that flavonoids have the useful property of preventing capillary bleeding and fragility in scorbutic animals. Despite extensive studies in the 1950's (*2*), unequivocal proof for such a role on capillary permeability was not obtained; consequently interest in flavonoids waned (*3*). The question of their biological action, therefore, remains unanswered.

In the present report evidence is presented to show that flavonoids can exert an entirely different kind of biological effect—inhibiting aldose reductase. This enzyme has been involved in the formation of cataracts in diabetes and galactosemia (*4, 5*). The formation of sugar alcohols from sugars catalyzed by aldose reductase appears to initiate the cataractous process (*5*); inhibitors of aldose reductase are effective in delaying the onset of sugar cataracts (*5–7*). Further interest in aldose reductase and its inhibitors stems from the possibility that the enzyme may also be involved in the manifestation of some other secondary diabetic complications, such as neuropathy and angiopathy (*8*). The basis of this study was the possibility that flavonoids may be more effective than other known inhibitors of aldose reductase.

The inhibitory action of various flavo-

Table 1. Inhibition of lens aldose reductase by various compounds. The numbers indicate percentage of inhibition of the aldose reductase activity as compared to controls when the reaction was carried out in the absence of inhibitors. The number of experiments in each case was at least four. The standard deviation of the results was within 5 percent. All the compounds tested inhibited the enzyme activity almost completely at $10^{-4}M$. Abbreviations: TMG, tetramethylene glutaric acid; AY-22,284, 1,2-dioxo-1H-benz-(de)-isoquinoline-2(3H) acetic acid.

Inhibitors	Percentage of inhibition at the following concentrations		
	$10^{-5}M$	$10^{-6}M$	$10^{-7}M$
TMG	82	35	0
AY-22,284	90	40	0
Quercetin	83	60	15
Rutin	95	20	10
Quercitrin	95	88	55
Myricitrin	100	75	35
Morin	75	0	0
Hesperetin	50	0	0
2-Carbethoxy-5,7-dihydroxy-4′-methoxy-isoflavone	77	0	0
Robinin	56	0	0

noids was tested on partially purified rat lens enzyme. The methods used in enzyme purification and determination of its activity were similar to those previously described (*9*). Flavonoids used are commercially available; chemical determinations were those used previously (*7, 10*).

Table 1 lists the inhibitory activity of various compounds studied, and the structures of the flavonoids are given in Fig. 1. All the flavonoids tested have significant inhibitory activity. In fact, quercetin, quercitrin, and myricitrin were much more effective as inhibitors of aldose reductase than either AY-22,284 [1,2-dioxo-1H-benz-(de)-isoquinoline-2(3H) acetic acid] or tetramethylene glutaric acid, the previously known inhibitors. The simplest of the flavonoids shown in Fig. 1 are the aglycones. Since quercetin is more potent an inhibitor of aldose reductase than morin, the ortho orientation of the hydroxyls in positions **3′** and **4′** of ring C rather than a meta orientation is more favorable to inhibitory activity. Hesperetin, in which there is the absence of unsaturation and of the hydroxyl group from ring B, and the para-hydroxyl group in ring C is methylated, has much lower activity than the other aglycones.

The glycoside of a flavone may have a higher or lower inhibitory activity than its parent aglycone, depending upon the nature of the glycosylating sugar. For example, quercitrin, the 3-L-rhamnoside, has a higher activity than its aglycone quercetin. On the other hand, the rutinoside of quercetin, rutin, is less active than the aglycone. An additional hydroxyl group in ring C, as in myricitrin, appears to lower the inhibitory activity only slightly. So far we have examined only a limited number of flavone derivatives and it is possible that some other flavonoids may be more active as aldose reductase inhibitors than those tested here.

The inhibitory activity of flavonoids on aldose reductase was next tested in the intact rat lenses cultured in a medium with a high xylose content as described previously (*10*). Xylose, along with galactose and glucose, is a cataractogenic sugar (*5*). When the lenses were incubated in xylose for 4 hours, substantial quantities of xylitol accumulated (*10*). Inclusion of quercitrin in the medium at a concentration of $10^{-4}M$ reduced the amount of polyol (xylitol) accumulation by 80 percent of the control. Under similar conditions AY-22,284 reduced the polyol accumulation by 40 percent of the control; at 10^{-5} and $10^{-6}M$, AY-22,284 was ineffective while quercitrin reduced polyol synthesis by about 30 and 20 percent, respectively. These results again emphasize the point that quercitrin is a much more effective aldose reductase inhibitor than AY-22,284. Both inhibitors, however, do not readily penetrate the lens permeability barrier since higher concentrations were required in lens culture to cause a similar magnitude of inhibition as that observed in the purified enzyme experiments.

Since flavonoids are relatively nontoxic (*11*), the present finding that some of them are the most potent inhibitors of aldose reductase known so far suggests that they may be useful in preventing the onset of diabetic or galactosemic cataracts as well as an aid in assessing the role of aldose reductase in diabetic neuropathy and angiopathy.

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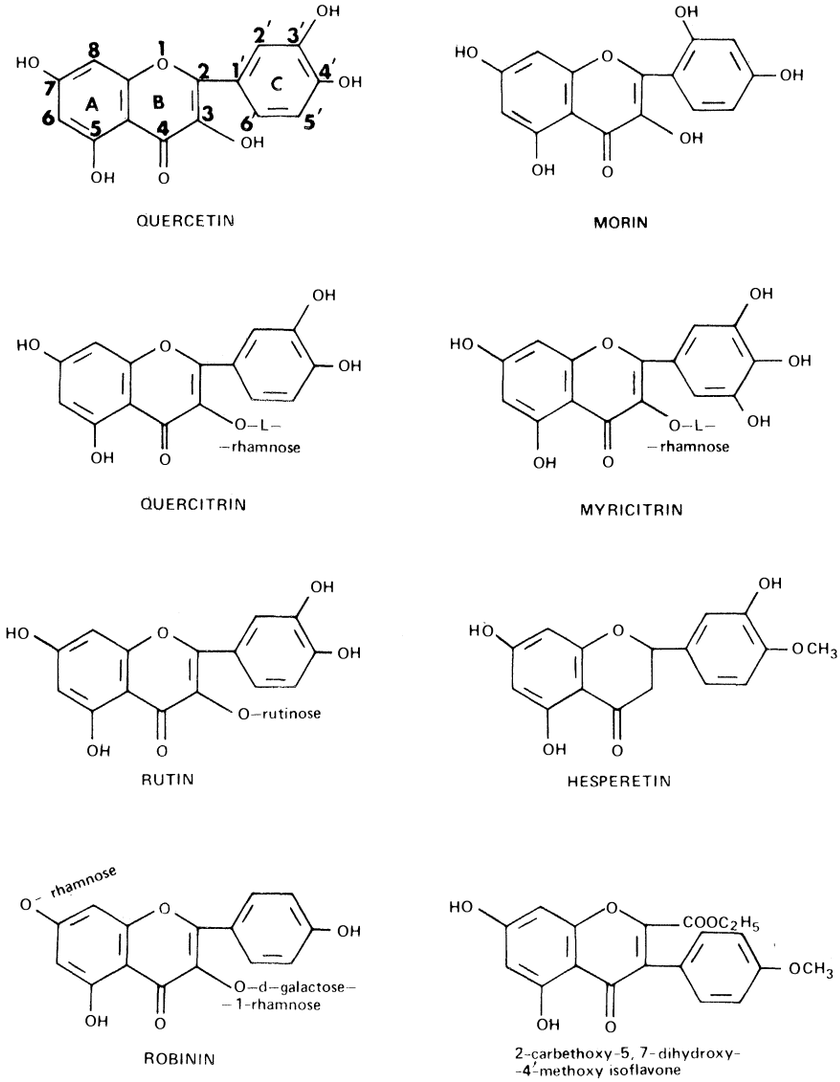

Fig. 1. Structures of the flavonoids with the aldose reductase inhibitory activity. The letters A, B, and C assigned to the rings and the boldface numbers assigned to positions in quercetin are applicable to other compounds also.

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