[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

Interconversion of Chalcones and Flavanones of a Phloroglucinol-type Structure

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Naturally occurring flavanone glycosides with a phloroglucinol-type structure were converted to the corresponding chalcone glycosides. The method of preparation and the properties of the latter compounds are described. Some preliminary evidence was obtained for the existence of an enzyme, "flavanone synthease," which converts phloroglucinol-type chalcone glycosides to flavanone glycosides. It is postulated that a chalcone glycoside may in certain cases serve as an intermediate in the biogenesis of a flavanone glycoside.

In a previous paper[1] it was shown that the flavonoid pigments of *Coreopsis tinctoria* consist of a chalcone, a flavanone and a benzalcoumaranone derivative of corresponding structure. It was found that the chalcone, marein, is oxidized easily to the corresponding aurone, maritimein, but at the same time it has a great tendency to isomerize into the related flavanone, flavanomarein. The unusual behavior shown by marein suggests that there may be a close relationship among the three types of flavonoid pigments. This finding, in addition to a number of reports in the literature which are summarized below, prompted us to investigate the interconversion of chalcones and flavanones; flavanone glycosides with a phloroglucinol type of structure, *viz.*, naringin, poncirin, hesperidin and isosakuranin, were used for this purpose.

A majority of the naturally occurring flavanones have a phloroglucinol structure. However, no chalcone possessing a phloroglucinol or a 2′,6′-dihydroxy structure with free hydroxyl groups in the 2′- and 6′-positions is known in nature. Salipurposide found in *Salix purpurea*[2] is fairly stable in the chalcone form (isosalipurposide); it has a glucosidoxy residue in place of one of the *o*-hydroxyl groups. Other examples of the co-existence of a flavanone with a corresponding chalcone have been reported by Seshadri.[3]

Narasimhachari and Seshadri,[4] in their study of a number of substituted flavanones, found that naringenin, isosakuranetin, naringenin 4′,7-dimethyl ether, etc., dissolve readily in cold 10% sodium hydroxide and are precipitated unchanged by acidification, whereas 5-methoxy- and 5,7-dimethoxyflavanone dissolve in this reagent only on warming and give only the corresponding 2′-hydroxy chalcones on acidification. From these experiments they pointed out that when the 5-hydroxyl group is present in the flavanone, the equilibrium in the chalcone–flavanone isomerization is shifted in favor of the flavanone because of hydrogen-bonding stabilization between the 5-hydroxyl group and the carbonyl.[4]

Of particular interest are optically active flavanones, a few of which have been found in nature.[5]

In 1942, Zemplén and co-workers[6] synthesized an isosakuranetin glucoside (m.p. 214°, $[\alpha]_D$ −73.4° in pyridine) through its chalcone form and ascribed to it the structure of a 7-glucoside. Natural isosakuranetin-7-glucoside (m.p. 190°, $[\alpha]^{13}_D$ −41.4° in 60% acetone) was isolated from the wood of *Prunus donarium* var. *spontanea* by Hasegawa and Shirato[7] who attributed the discrepancy in the two preparations to the difference in the optical activity of the aglycon.

Wawra and Webb[8] reported the preparation of hesperidin chalcone by dissolving hesperidin in cold alkali; upon neutralization the chalcone crystallized. Their preparation melted at 251–252° and showed ultraviolet absorption maxima at 332 and 308 mμ. Almost the same procedure was repeated by the author, giving pale yellow crystals, which did not show any of the absorption characteristics of chalcones. It will be shown below that, under the comparatively mild conditions used by Wawra and Webb, the equilibrium, flavanone ⇄ chalcone, is not shifted to the right.

The existence in nature of optically active flavanones suggests that an enzyme may be involved in the chalcone–flavanone isomerism; various sources were tested for the presence of such an enzyme.

Experimental[9]

Isosakuranin from Poncirin.—Poncirin isolated from *Poncirus trifoliata*[5e] was partially hydrolyzed by refluxing a solution of 2 g. in 25 ml. of 50% ethanol and 0.5 ml. of 20% hydrochloric acid for 3 hr. Paper chromatography revealed that the poncirin had almost disappeared, while the isosakuranetin spot was still weak and a spot of intermediate R_f was most prominent. In agreement with this finding, a marked spot of rhamnose was detected, whereas glucose showed a weak spot. When the hydrolyzate was allowed to stand overnight, colorless needles of isosakuranin separated almost completely; yield 0.4 g. The isosakuranin was repeatedly recrystallized from 50% ethanol; m.p. 172–178°, $[\alpha]^{20}_D$ −48.4° (*c* 0.25, 90% ethanol).

Anal. Calcd. for $C_{28}H_{34}O_{10}\cdot 1.5H_2O$: C, 55.57; H, 5.72; H_2O, 5.69. Found: C, 55.76; H, 5.43; H_2O, 5.60.

Chalcones.—The following procedure exemplifies the preparation of the chalcones from the corresponding flavanones:

(A) **Isosakuranin.**—Isosakuranin (1 g.) was heated with 2 g. of potassium hydroxide and 4 ml. of water on a boiling water-bath for 2 minutes. On cooling, the mixture was acidified with 20% hydrochloric acid. The resulting orange-yellow crystalline precipitate was recrystallized from 40 ml. of 30% ethanol as yellow needles, m.p. 172–175°, yield 0.55 g.

(1) M. Shimokoriyama, THIS JOURNAL, **79**, 214 (1957).

(2) G. Zemplén, R. Bognár and I. Székely, *Ber.*, **76**, 386 (1943).

(3) T. R. Seshadri, *Sci. Proc. Roy. Dublin Soc.*, **27**, 77 (1956).

(4) N. Narasimhachari and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **27**, 223 (1948); *C. A.*, **44**, 1493*d* (1950).

(5) (a) S. Fujise, *Sci. Papers Inst. Phys. Chem. Res. (Tokyo)*, **11**, 111 (1929); S. Fujise and T. Kubota, *Ber.*, **67**, 1905 (1934); (b) H. R. Arthur, *J. Chem. Soc.*, 3740 (1955); (c) H. Erdtman, *Svensk Papperstidn.*, **46**, 226 (1943); *C. A.*, **37**, 5862[8] (1943); (d) H. R. Arthur, W. H. Hui and C. N. Ma, *J. Chem. Soc.*, 632 (1956); (e) S. Hattori, M. Hasegawa and M. Shimokoriyama, *Acta Phytochim.*, **14**, 1 (1944); (f) C. Sannié and A. Sosa, *Fruits outre mer*, **4**, 4 (1949); *C. A.*, **43**, 4669*h* (1949).

(6) G. Zemplén, R. Bognár and L. Mester, *Ber.*, **75**, 1432 (1942).

(7) M. Hasegawa and T. Shirato, THIS JOURNAL, **77**, 3557 (1955).

(8) C. Z. Wawra and J. L. Webb, *Science*, **96**, 302 (1942); *C. A.*, **36**, 7090[5] (1942).

(9) All melting points are not corrected.

Anal. Calcd. for $C_{22}H_{24}O_{10}\cdot 1.5H_2O$: C, 55.57; H, 5.72; H_2O, 5.69. Found: C, 55.80, 55.52; H, 5.93, 6.01; H_2O, 5.80.

(B) **Poncirin.**—The reaction mixture, obtained by similar treatment of 1 g. of poncirin, was acidified to *p*H 2. Treatment of the oily precipitate with ethyl acetate gave crystals which, upon three recrystallizations from 50% ethanol, yielded yellow needles, m.p. 195–205°, yield 0.45 g.

Anal. Calcd. for $C_{28}H_{34}O_{14}\cdot H_2O$: C, 54.90; H, 5.92; H_2O, 2.94. Found: C, 55.02; H, 5.64; H_2O, 3.00.

(C) **Naringin.**—Naringin (1.3 g.) isolated from the peel of *Citrus natsudaidai*[10] was treated as described for poncirin. The chalcone was obtained as yellow needles, m.p. 185–200°, yield 0.7 g.

Anal. Calcd. for $C_{27}H_{32}O_{14}\cdot H_2O$: C, 54.18; H, 5.73; H_2O, 3.01. Found: C, 54.15; H, 5.51; H_2O, 3.20.

(D) **Hesperidin.**—Yellow needles of the chalcone were obtained from hesperidin isolated from *Citrus nobilis* peel. The hesperidin chalcone melted at 180° and the melted mass, gradually losing color, solidified as colorless crystals which melted at 250°, the melting point of hesperidin.

Anal. Calcd. for $C_{28}H_{34}O_{15}\cdot 2.5H_2O$: C, 51.30; H, 6.00; H_2O, 6.87. Found: C, 51.19; H, 5.49; H_2O, 7.12.

These chalcones dissolved instantly in cold, dilute aqueous alkali giving an orange color; their ethanolic solutions gave a brown color with ferric chloride.

Preparation of Racemic Flavanones from Chalcones. (A) **Isosakuranin.**—Isosakuranin chalcone (1.0 g.) was heated on a water-bath with 0.3 g. of sodium acetate and 3 ml. of water according to the method of Zemplén.[6] The separation of colorless crystals of the flavanone, which occurred after a few minutes, was almost complete in 15 minutes. The product was recrystallized from 50% ethanol; m.p. 210–214°, $[\alpha]^{20}D$ −39.2° (*c* 0.25, 90% ethanol).

Anal. Calcd. for $C_{22}H_{24}O_{10}\cdot 1.5H_2O$: C, 55.57; H, 5.72; H_2O, 5.69. Found: C, 55.94; H, 5.82; H_2O, 5.42.

(B) **Poncirin.**—A mixture of 0.1 g. of the chalcone, 1 ml. of 50% ethanol and 0.1 ml. of McIlvaine buffer solution of *p*H 7 was heated on a water-bath for 3 minutes. The reaction mixture was extracted with ethyl acetate, the extract evaporated *in vacuo* and the residue crystallized from absolute ethanol; yield 40 mg. The crystals softened at 160° and melted at 170–175°, $[\alpha]^{20}D$ −82.5°; natural poncirin, $[\alpha]^{20}D$ −93.8° (*c* 0.8, 90% ethanol).

(C) **Naringin.**—The chalcone (0.1 g.) was heated with 1 ml. of 50% ethanol and 0.1 ml. of McIlvaine buffer solution (*p*H 6). The mixture was added to an equal volume of water and allowed to stand overnight. The crystals which separated were recrystallized from 20% ethanol as colorless needles, m.p. 80–83°, yield 80 mg., $[\alpha]^{20}D$ −72.1°; natural naringin, $[\alpha]^{20}D$ −82.0° (*c* 1, 90% ethanol).

(D) **Hesperidin.**—Racemic hesperidin, prepared by the procedure described for racemic naringin, was recrystallized from pyridine as colorless needles, m.p. 250°.

Enzymatic Hydrolysis of Isosakuranin with Emulsin.—Isosakuranin (0.1 g.) suspended in 6 ml. of water was added to 6 ml. of a solution containing 60 mg. of emulsin, prepared from Japanese apricot. The mixture was covered with toluene and incubated at 30°. The hydrolysis was almost complete in three days. The aglycon was filtered and recrystallized from dilute ethanol; m.p. 170°, reported[7] m.p. 170°; $[\alpha]^{20}D$ −13.8° (*c* 1, 90% ethanol).

Anal. Calcd. for $C_{16}H_{14}O_5\cdot {}^1/_2H_2O$: C, 65.08; H, 5.12. Found: C, 65.35; H, 5.36.

The filtrate, separated from the aglycon, was evaporated to about 1 ml. and chromatographed with 1-butanol–acetic acid–water (4:1:1) as the solvent and a benzidine solution as the developer. The concentrate gave a single spot, which corresponded to glucose (R_f 0.17).

When racemized isosakuranin was hydrolyzed by emulsin, an optically inactive isosakuranetin (m.p. 190°) was obtained.

Stability of Chalcones at Various *p*H Values.—The behavior of chalcones in dilute ethanolic solution was studied at various *p*H's. The times required for the complete decolorization of the buffered solutions, *i.e.*, for the complete conversion of the chalcone to flavanone, are given in Table I.

(10) M. Tsujimura, *Bull. Inst. Phys. Chem. Res. (Tokyo)*, **6**, 1111 (1927).

TABLE I

DECOLORIZATION TIME IN MINUTES OF CHALCONES AT VARIOUS *p*H VALUES

To 0.2 ml. of ethanolic solution of chalcones (0.01 *M*) were added 0.5 ml. of water and 0.3 ml. of McIlvaine buffer solution (22°) ppt and PPT indicate the presence of crystals of chalcone and flavanone, respectively.

Chalcone	*p*H 3	4	5	6	7	8
Isosakuranin	ppt ∝	ppt ∝	ppt ∝	70	PPT 22	PPT 15
Poncirin	ppt ∝	ppt ∝	ppt ∝	65	22	16
Naringin	ppt ∝	∝	PPT 400	PPT 70	PPT 20	PPT 15
Hesperidin	∝	∝	PPT 420	PPT 80	PPT 21	PPT 15

This isomerization reaction also was followed by measuring the changes in the absorption of buffered chalcone solutions at 370 mμ with a spectrophotometer against an appropriate buffer blank; the data are recorded in Fig. 1. This isomerization is a monomolecular reaction. The rate constant *k* was calculated from the formula

$$k = \frac{2.303}{t} \log \frac{E_0}{E_t}$$

where E_0 and E_t represent the optical densities of the solution at 370 mμ at zero time and at *t* minutes, respectively. The following values were obtained for *k*: 0.150 at *p*H 7, 0.055 at *p*H 6, 0.018 at *p*H 5, 0.001 at *p*H 4 and 0.000 at *p*H 3.

Preparation of an Enzyme Solution from the Peel of *Citrus aurantium*.—One hundred grams of the peel was macerated twice with 300 ml. of cold ethanol, and the almost colorless powder obtained was then extracted with 300 ml. of water. The aqueous solution was mixed with one-tenth volume of a saturated ammonium sulfate solution and then centrifuged. The supernatant was saturated with ammonium sulfate and the resulting precipitat was filtered, redissolved and reprecipitated by the same procedure as above. The precipitate was then dissolved in 20 ml. of water, dialyzed and filtered. In order to test the activity of the "flavanone synthease," 10 mg. of the chalcone glycoside suspended in 0.5 ml. of water (in some cases buffered with McIlvaine solution) was added to 0.5 ml. of the above enzyme solution. The mixture was covered with toluene and incubated at 30°. A blank test was always run on the same quantity of the chalcone suspended in 1 ml. of water. The conversion of the chalcone to flavanone was readily detected by disappearance of the chalcone or by appearance of the resulting flavanone in crystals.

TABLE II

THE ACTIVITY OF "FLAVANONE SYNTHEASE" FROM VARIOUS SOURCES

Species	Tissue	Relative activity[a]
Citrus aurantium	Peel	+++
	Leaf	−
C. natsudaidai	Peel	++
	Leaf	−
C. junos	Peel	±
C. nobilis	Peel	±
C. pseudoparadisi	Peel	±
Poncirus trifoliata	Peel	+
	Leaf	−
Cosmos sulphureus	Ray	−
	Leaf	−
Coreopsis lanceolata	Ray	−
	Leaf	−
C. tinctoria	Ray	−
	Leaf	−
Dahlia variabilis (a white variety)	Ray	−
	Leaf	−

[a] + + + denotes almost complete conversion of the chalcone glycoside (10 mg. in 0.5 ml. of water plus 0.5 ml. of enzyme solution) in 3 days, + + in 5 days, + signifies partial conversion, ± slight activity and − no activity.

Some other plant materials were tested for enzymatic activity in the same way; the results are summarized in Table II.

No isomerization reaction was observed if the enzyme solution had been boiled for about 10 minutes. The activity also was inhibited completely by mercuric chloride (0.002*M*) but not affected by potassium cyanide (0.002*M*). The cyclization did not occur at *p*H 3–4. The spontaneous reaction, however, occurs so readily at *p*H 5 and so rapidly at *p*H 6–7 that it was impossible to find the optimum *p*H for the enzyme. If an unbuffered aqueous suspension was used, the chalcone was almost perfectly stable during a week's incubation, owing to its insolubility in water. When these chalcones were subjected to the oxidizing action of the chalconase[11] in *Cosmos* or *Coreopsis*, the conversion into the corresponding aurones was not, however, observed. As chromatographic analysis showed, the marein from *Coreopsis tinctoria*[1] and the coreopsin from *Cosmos sulphureus*[12] were not isomerized by the "flavanone synthease"; flavanomarein[1] and flavanocoreopsin, obtained as described below, served as controls.

Enzymatic Resynthesis of Flavanones. (A) Naringin.—A mixture of the chalcone (0.5 g.), suspended in 25 ml. of water, and 25 ml. of the enzyme solution prepared from the peel of *Citrus aurantium* was covered with toluene and incubated at 30°. The reaction was complete in 3–4 days; by that time no yellow crystals of chalcone remained and colorless needles of naringin had appeared. The mixture was cooled in a refrigerator overnight, and the flavanone was filtered and recrystallized from 20% ethanol; m.p. 80–83°, $[\alpha]^{20}D$ −74.3° (*c* 1, 90% ethanol).

(B) **Isosakuranin.**—Isosakuranin prepared from the chalcone in the same way melted at 175–190°, $[\alpha]^{20}D$ −43.0° (*c* 0.25, 90% ethanol).

(C) **Poncirin.**—Poncirin chalcone, which had been incubated with the enzyme, gave an almost clear, pale brown solution which was extracted with ethyl acetate. The extract was evaporated *in vacuo* and the residue crystallized twice from absolute ethanol to yield colorless crystals of the flavanone, m.p. 160–170°, $[\alpha]^{11}D$ −84.2° (*c* 0.8, 90% ethanol).

Flavanocoreopsin.—Coreopsin (1.2 g.) was heated on a water-bath with 3.6 g. of sodium acetate and 18 ml. of water for about 2 hr. Then the solution was slightly acidified with a few drops of 20% acetic acid and extracted with ethyl acetate. The acetate solution was evaporated and the residue was dissolved in 10 ml. of ethanol. The pale yellow needles which separated were recrystallized repeatedly from ethanol to yield almost colorless needles which melted at 166–168° and gave a green color with ferric chloride. The flavanocoreopsin gave an R_f value of 0.62 (1-butanol–acetic acid–water, 4:1:2; Whatman No. 1; 20°); that of coreopsin was 0.54.

Anal. Calcd. for $C_{21}H_{22}O_{10}\cdot H_2O$: C, 55.75; H, 5.35; H_2O, 3.98. Found: C, 55.81; H, 5.84; H_2O, 4.04.

The conversion did not, however, occur under the conditions used for the cyclization of phloroglucinol-type chalcones. Flavanocoreopsin could not be detected even in trace amounts in the fresh extract of the ray flowers of *Cosmos sulphureus*.

Discussion

All of the chalcones obtained from phloroglucinol-type flavanone 7-glycosides as described above are fairly stable even when dissolved in ethanol, either concentrated or dilute. They are readily soluble in strong ethanol and recrystallizable from dilute ethanol. That these substances have a chalcone structure is indicated by the following experiments and considerations: They are bright yellow, while the original as well as the reconverted flavanones are colorless. They dissolve instantly in cold, dilute aqueous alkali giving an orange color. They gave a brown color with ferric chloride, whereas the flavanones studied in the present work gave a purple color. The chalcones gave well-separated yellow spots on a paper chromatogram, not accompanied by even a faint spot of corresponding flavanones; while the mother liquor of recrystallization, after being allowed to stand a few days, gave both spots. The R_f values are shown in Table III. Their ultraviolet absorption spectra, two examples of which are shown in Fig. 2 with one typical flavanone curve, confirm the chalcone structure assigned to these compounds. An imperfect chalconization or careless treatment gave a pale yellow preparation which had an absorption curve between that of the chalcone and the flavanone.

(11) M. Shimokoriyama and S. Hattori, THIS JOURNAL, **75**, 2277 (1953).

(12) M. Shimokoriyama and S. Hattori, *ibid.*, **75**, 1900 (1953).

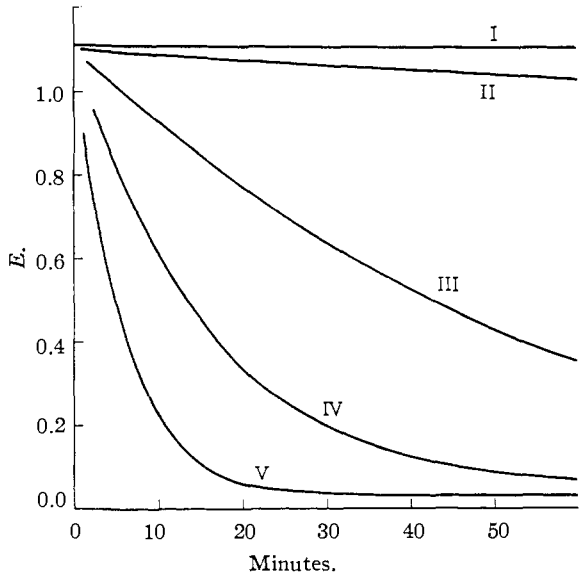

Fig. 1.—Isomerization of naringin chalcone to the flavanone at various *p*H values (22°). To 1.0 ml. ethanolic solution of the chalcone (0.003 *M*) were added 1.0 ml. of McIlvaine buffer solution and 8.0 ml. of water. I, II, III, IV and V indicate the reaction curves measured at *p*H 3, 4, 5, 6 and 7, respectively.

TABLE III

R_f VALUES AND COLORS OF CHROMATOGRAPHED CHALCONES AND FLAVANONES (BuOH–HOAc–H_2O, 4:1:2; 13°; WHATMAN No. 1)

	R_1	Untreated[a] V	Untreated[a] UV	NH_3 V	NH_3 UV
Isosakuranin chalcone	0.79	Y	B	OY	Bk
Isosakuranin	.76	C	pY	C	Y
Poncirin chalcone	.68	Y	B	OY	Bk
Poncirin	.61	C	pY	C	Y
Naringin chalcone	.64	Y	B	OY	Bk
Naringin	.54	C	pY	C	Y
Hesperidin chalcone	.38	Y	B	OY	Bk
Hesperidin	.36	C	pY	C	Y

[a] B brown, Bk black, C colorless (not visible), O orange, p pale, UV ultraviolet light, V visible light, Y yellow.

The stability of the chalcones at various *p*H values exceeded our expectation. An equilibrium may exist in the interchange, chalcone ⇄ flavanone, and this equilibrium is shifted to the left in an alkaline medium and to the right in an acid medium. Our study of the behavior of chalcones in a dilute ethanolic solution of various *p*H values (Table I and Fig. 1) is noteworthy in two respects. In the

first place, the cyclization is a monomolecular reaction. Secondly, the reaction took place rapidly in alkaline and neutral media; the chalcones were fairly stable in weak acid and extremely stable at *p*H 3. These facts should be kept in mind in the preparation and purification of chalcones; careful neutralization gives either a mixture of chalcone and flavanone or flavanone alone.

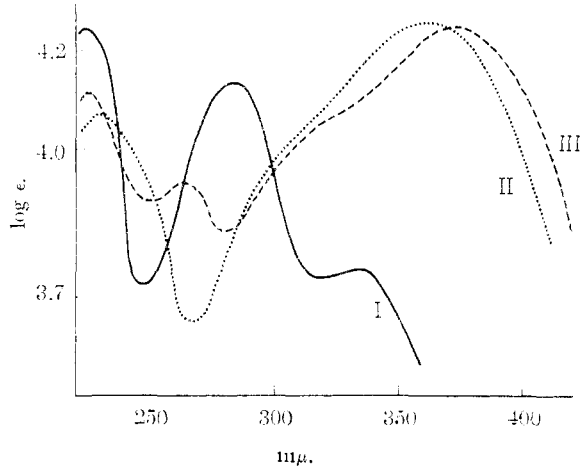

Fig. 2.—Ultraviolet absorption spectra: I, pocirin and isosakuranin; II, poncirin chalcone and isosakuranin chalcone; III, hesperidin chalcone (in 98% ethanol).

The existence of optically active flavanones in nature suggested that if a chalcone was produced as a precursor in the biosynthesis of a flavanone, an enzyme may be involved in the chalcone-flavanone isomerism. The fact that the phloroglucinol-type chalcone glycosides are unstable in the *p*H range prevailing in living cells and form optically inactive flavanones supports this hypothesis. Some evidence for the existence of such an enzyme, "Flavanone synthease," was obtained. The aqueous, cell-free extract of the peel of *C. aurantium* proved to be the most active preparation. The enzyme in the peel of *Citrus* fruits acted in almost the same way on all the phloroglucinol-type chalcones tested, but it did not seem to exert any effect on the coreopsin from *Cosmos sulphureus* and the marein from *Coreopsis tinctoria*. No activity was detected in the ray and the leaves of *Cosmos*, *Coreopsis* and *Dahlia*.

Few optically active forms of flavanone glycosides have been found in nature. By the usual procedure for the hydrolysis of a glycoside, however, some racemization always takes place. Consequently, enzymatic hydrolysis is the best method for obtaining an optically active aglycon from a flavanone glycoside. Isosakuranin prepared from poncirin was hydrolyzed readily by an emulsin from Japanese apricot to give optically active isosakuranetin; Hasegawa and Shirato[7] obtained the same results with isosakuranin from *Prunus*. When isosakuranin chalcone was recyclized, it gave isosakuranin (m.p. 210–214°) which is regarded as identical with the isosakuranin described by Zemplén and co-workers.[6] A mixture of racemic isosakuranin and the original preparation gave on recrystallization a preparation which melted between 180 and 200°. Enzymatically resynthesized isosakuranin melted in the same temperature range, a finding which suggests that it contains some optically active isosakuranetin. The specific rotation values of these preparations are in good accord with these facts: $[\alpha]^{20}D$ for isosakuranin prepared from poncirin was −48.4°, that of the completely racemized glucoside was −39.2°, and that of the enzymatically recyclized preparation −43.0°. Although the isosakuranin from poncirin is, of course, partially racemized during hydrolysis and purification, the isosakuranin enzymatically resynthesized from the chalcone is fairly rich in optically active isosakuranetin.

In the case of poncirin and naringin, there was almost no difference between the melting points of the completely racemized compounds obtained *via* the chalcone and those of the original flavanones. However, the specific rotation powers of the recyclized flavanones were about 10° less than those of the original compounds, a difference which indicates that the aglycon occurs in the plant in an optically active form; it would necessarily be partly racemized during extraction and purification. The specific rotation values of the enzymatically resynthesized glycosides indicate that the products contain optically active aglycon to some extent.

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