N,N'-Dimethylisopropanesulfinamide (III), **N,N'-dimethylmethanesulfinamide** (IV), and **N,N'-dimethylpropanesulfinamide** (VI) were supplied by Dr. J. S. Luloff, Shulton Chemical Co., Clifton, N. J. These compounds had b.p. 66° (6 mm.), b.p. 55° (1.75 mm.), and b.p. 55° (1.1 mm.), respectively.

N,N'-Dimethyl-*p*-toluenesulfinamide (VII) has already been reported.[11]

N,N'-Dimethyl-*p*-toluenesulfonamide (VIII) was prepared according to the method of Klamann[22] and had m.p. 87–88°, lit. m.p. 86–87°.

N,N'-Diethyl-*p*-toluenesulfonamide (IX).—Addition of 7.3 g. (0.10 mole) of diethylamine in 50 ml. of ether to a solution of 3.8 g. (0.02 mole) of *p*-toluenesulfonyl chloride in 25 ml. of ether resulted in a slow precipitation of diethylamine hydrochloride. After 16 hr. at room temperature, the precipitate was filtered and the solution was concentrated to dryness *in vacuo*. The resulting crystalline residue was recrystallized from pentane to yield 4.3 g. of IX, m.p. 59–60°, lit.[17] m.p. 60°.

(22) D. Klamann, G. Hoffbauer, and F. Drahowzal, *Monatsh. Chem.*, **83**, 870 (1952).

Acknowledgment.—We wish to thank Dr. R. J. Highet, National Institutes of Health, Bethesda, Maryland, for determining the spectra reported in this study and for many helpful suggestions. Theoretical spectra were computed using the facilities of the National Institutes of Health. Our thanks is given also to Dr. J. S. Luloff, Shulton Chemical Company, Clifton, New Jersey, for supplying samples of sulfinamides.

Cyanogenesis in *Sorghum vulgare*. II. Mechanism of the Alkaline Hydrolysis of Dhurrin (*p*-Hydroxymandelonitrile Glucoside)[1,2]

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Dhurrin, the cyanogenetic glucoside of *Sorghum vulgare*, is alkali labile although not structurally related to any of the known classes of alkali-labile glycosides. A mechanism for the alkaline hydrolysis is proposed, involving the successive ejection, from the dhurrin anion, of the two groups attached to the benzyl carbon. As predicted by this mechanism, the rate of the decomposition is proportional to the concentration of the anion. The reaction is first order, with $k = 0.032$ min.$^{-1}$ at 25°. The glucosyloxy group is ejected first, then the cyanide. *p*-Methoxymandelonitrile β-D-glucopyranoside and *o*- and *p*-hydroxybenzyl β-D-glucopyranosides were prepared in solution and characterized. The *p*-methoxy derivative is stable to alkali; the hydroxybenzyl glucosides are alkali labile, but less so than dhurrin.

In the course of work on the isolation of dhurrin (**1**, *p*-hydroxy-L-mandelonitrile β-D-glucopyranoside) from *Sorghum vulgare*, we found that this glucoside, while showing no unusual instability in acid, was extremely labile to alkali.[1] This property of dhurrin has also been observed by Conn and co-workers.[3] Alkali-labile glycosides are not common; however, a considerable number are known, and according to Ballou[4] they can be grouped into three structural types: (a) glycosides of phenols, (b) glycosides of enols, and (c) glycosides of alcohols substituted in the β-position by a negative group. Since dhurrin does not belong to any of these three types, a study of the mechanism of its alkaline hydrolysis seemed to be of interest.

The long-known mandelonitrile glucosides such as amygdalin (D-mandelonitrile β-gentiobioside)[5] and prunasin (D-mandelonitrile β-D-glucopyranoside),[5] which have no substituents in the aromatic ring, undergo certain changes in alkaline solutions, but their glycoside linkages are not cleaved.[6] It is therefore clear that the hydroxyl group on the benzene ring of dhurrin is responsible for its alkali sensitivity. A consideration of the ways in which alkali could act on the dhurrin molecule led us to postulate a reaction sequence (this is shown in Scheme I), which is an elaboration of the mechanism for the base-catalyzed decomposition of the *p*-hydroxybenzyl halides and related compounds.[7] This mechanism involves the formation of a quinone methene intermediate by the ejection of a leaving group from the benzyl carbon of the substrate anion. When dhurrin is the substrate a second elimination is possible, after addition of hydroxide ion to the quinone methene, and the reaction could follow either of two pathways: A, ejection of a glucosyloxy anion (**4**) in the first step, or B, ejection of a cyanide ion in the first step. There seemed to be no *a priori* basis for favoring one of these pathways over the other. However, the over-all mechanism has the following features: (a) the rate of alkaline hydrolysis should be proportional to the concentration of the anionic form of dhurrin (*i.e.*, a plot of the rate of decomposition against pH should be identical with the dissociation curve); (b) the decomposition rate at any given pH should obey the first-order rate law; (c) if the phenolic hydroxyl is converted into a nonionizing group, such as methoxyl, the resulting glucoside should be stable in alkaline solution; and (d) the bond cleaved should be the one between the glycosidic oxygen and the aglycone. The present paper describes experiments on the behavior of dhurrin and related compounds in which these predictions were tested, and the sequence of appearance of the hydrolysis products was determined.

(1) Paper I of this series: C.-H. Mao, J. P. Blocher, L. Anderson, and D. C. Smith, *Phytochemistry*, in press.

(2) Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(3) T. Akazawa, P. Miljanich, and E. E. Conn, *Plant Physiol.*, **35**, 535 (1960).

(4) C. E. Ballou, *Advan. Carbohydrate Chem.*, **9**, 59 (1954).

(5) W. Karrer, "Konstitution und Vorkommen der Organischen Pflanzenstoffe," Birkhäuser Verlag, Basel, 1958, p. 949.

(6) F. K. Beilstein, "Handbuch der Organischen Chemie," Vol. XXXI, 4th Ed., 1919, pp. 238 and 400.

(7) L. J. Filar and S. Winstein, *Tetrahedron Letters*, **No. 25**, 9 (1960).

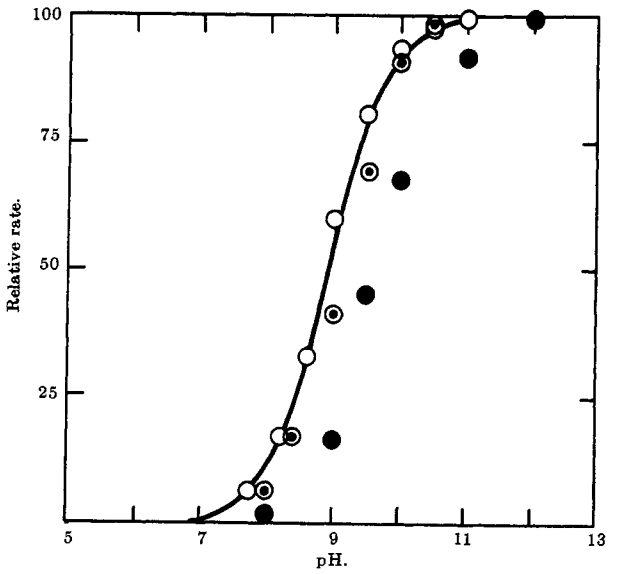


Figure 1.—Rates of decomposition of dhurrin at various pH values (points), and the dissociation curve of dhurrin (solid line): O, sodium bicarbonate–carbonate buffer; ⊙, sodium borate + potassium chloride; ●, sodium phosphate–borate.

Results and Discussion

Knowledge of the ultraviolet spectra of the compounds studied was required both for their characterization and for kinetic experiments. The pertinent data, obtained during the present work, are assembled in Table I. Values for some of the compounds have previously appeared in the literature, and in all these cases our values are in satisfactory agreement.

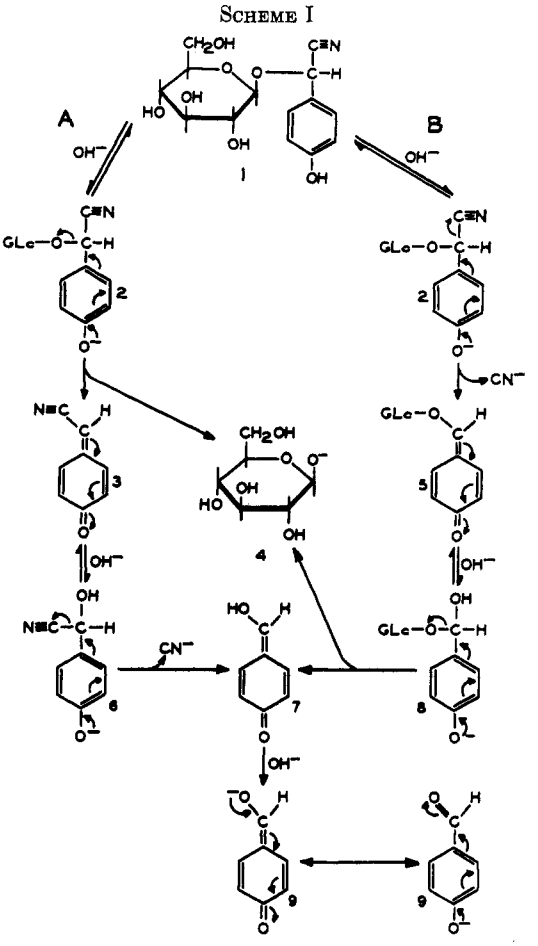
TABLE I

ULTRAVIOLET ABSORPTION MAXIMA OF HYDROXYBENZYL GLUCOSIDES AND RELATED COMPOUNDS

Compound	—Undissociated form— λ_{max}, mμ	A_m	—Ionized form— λ_{max}, mμ	A_m
Dhurrin	230	1.07×10^4	255	1.51×10^4
p-Hydroxybenzaldehyde	284	1.53×10^4	330	2.74×10^4
p-Methoxymandelonitrile β-D-glucoside	230	...	...	...
Anisaldehyde	284	1.61×10^4	284	1.61×10^4
p-Hydroxybenzyl β-D-glucoside	222	7.9×10^3	244	1.2×10^4
p-Hydroxybenzyl alcohol	222	7.87×10^3	244	1.23×10^4
o-Hydroxybenzyl β-D-glucoside	273	1.9×10^3	294	3.8×10^3
o-Hydroxybenzyl alcohol	273	1.93×10^3	294	3.84×10^3

The relative rates of hydrolysis of dhurrin at various pH values in the range 7.6–12 were studied by spectrophotometric determination of the initial velocity of the reaction. The quantity actually measured was the concentration of the final product, *p*-hydroxybenzaldehyde, which has an absorption maximum at 330 mμ in alkali, well separated from the alkaline peak of dhurrin at 255 mμ (Table I). In principle, it would have been simpler to follow the rate of disappearance of dhurrin, but this would have meant working in extremely dilute solutions. At the lower pH values, the plots of 330-mμ absorption *vs.* time showed a lag period of up to 5 min., because the decomposition of the intermediate (*p*-hydroxymandelonitrile, **6**, see below)

SCHEME I



is not instantaneous. The curves became linear after approximately 5 min., but even so there is some uncertainty in the rates determined at pH <9. The fact that the rate of decomposition of *p*-hydroxymandelonitrile increases rapidly with increasing pH[8] explains the absence of a lag period at the higher pH values.

A complete set of measurements was made in each of three standard buffer systems. The results are plotted in Figure 1, together with the dissociation curve of dhurrin.[1] It may be seen that, in sodium bicarbonate–carbonate buffer and sodium borate buffer, the rate of decomposition closely follows the dissociation curve, in accordance with the hypothesis that the decomposing species is the anion. In sodium phosphate–borate buffer, the values are smaller than in the other buffers. Apparently, phosphate ion has an inhibitory effect on the decomposition reaction.

The kinetics of the hydrolysis of the dhurrin anion were further studied in 0.067 *N* sodium hydroxide, in which dhurrin is completely dissociated. Figure 2 records the results of one of the kinetic runs, and shows that the over-all reaction obeys the first-order law to 75% decomposition. The rate constant, obtained by averaging the slopes of the log plots from nine runs, is 0.032 $min.^{-1}$ at 25°; the half-life of the reaction at this temperature is 21 min.

As a means of blocking the ionization of dhurrin, methylation was chosen. *p*-Methoxymandelonitrile β-D-glucopyranoside was prepared in solution and characterized as described in the Experimental section.

(8) Colette Bové and E. E. Conn, *J. Biol. Chem.*, **236**, 207 (1961).

The ultraviolet spectrum of this compound was identical with that of undissociated dhurrin (λ_{max} 230 mμ), and was not affected by the addition of alkali. The compound was completely stable to a copper reagent which has pH 9.6 (Somogyi)[9] for 15 min. at 100°. By comparison dhurrin gave, in 5 min., the amount of cuprous oxide corresponding to its total glucose. Since several minutes are required for the glucose to reduce the copper reagent, the decomposition of the dhurrin must have been complete in the first minute or two.

In the acid hydrolysis of a glycoside, the cleavage almost always occurs between the glycosidic oxygen and the anomeric carbon of the sugar, but in alkaline hydrolysis cleavage may take place on either side of the glycosidic oxygen depending on the electron distribution in the molecule.[10] A preliminary experiment to study the position of cleavage by the H_2O^{18} technique showed that, under the alkaline conditions required, the oxygen of water exchanged with the carbonyl oxygen of *p*-hydroxybenzaldehyde so fast as to render the method useless. Alkaline methanolysis (barium methoxide) was therefore employed in the study.[11] In the methanolysis reaction, if the glycosidic oxygen remained with the mandelonitrile moiety (as a result of attack by methoxide ion on the anomeric carbon of the sugar), methyl α-D-glucopyranoside should be produced. If, on the other hand, the glycosidic oxygen remained with the sugar, free glucose should appear instead.

Paper chromatography of the methanolysis reaction mixture revealed the presence of glucose, fructose, and an unknown component, presumably dhurrinic acid,[12] resulting from the solvolysis of the nitrile function. No methyl glucoside was present, indicating that the bond between the glycosidic oxygen and the benzyl carbon of the mandelonitrile was the one cleaved in the methanolysis of the glucoside link. In view of the close analogy between alkaline methanolysis and hydrolysis in aqueous base, this result may be taken as evidence that the hydrolysis occurs in the same way. The fructose which appeared on the paper chromatogram was the expected product of the isomerization of glucose in the alkaline solution.

To test the alkali lability of simple hydroxybenzyl glucosides, *p*-hydroxybenzyl β-D-glucopyranoside and *o*-hydroxybenzyl β-D-glucopyranoside were prepared in solution, by enzymatic synthesis, and purified by paper chromatography. When *p*-hydroxybenzyl glucoside was heated with the Somogyi reagent for 15 min. in a boiling-water bath, the amount of cuprous oxide formed corresponded to complete liberation of the glucose. *o*-Hydroxybenzyl glucoside was 75% decomposed, as judged by this criterion, while benzyl β-D-glucopyranoside was stable. The two hydroxybenzyl glucosides appear to be comparable, in their alkali lability, with vanillyl methyl ether, which is fairly rapidly cleaved at 80° and pH 8–10.[13] The leaving tendencies of the glucosyloxy and methoxy groups are thus similar in this reaction, as in some other reactions of substituted sugars. The simple hydroxybenzyl glucosides are considerably more stable than dhurrin.

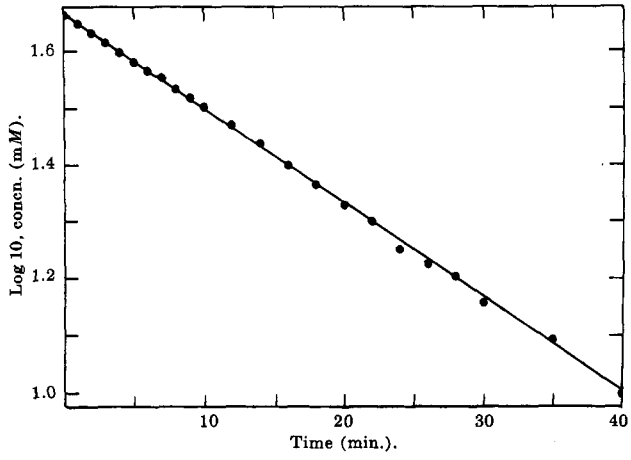


Figure 2.—Kinetics of decomposition of dhurrin in 0.067 *N* sodium hydroxide at 25°.

The close concordance between the predicted and actual outcome of the experiments discussed thus far gives strong support to the belief that the over-all mechanism of the alkaline hydrolysis of dhurrin is substantially that outlined in the introduction. No specific information was obtained on the mechanism of the decomposition of the simple *o*- and *p*-hydroxybenzyl glucosides, but it seems reasonable to assume that this too proceeds by the quinone methene pathway.

It has been suggested that decomposition *via* quinone methene intermediates is a characteristic property of hydroxybenzyl (*o*- and *p*-) compounds,[7] and the present data make it appear that this rule will apply to *o*- and *p*-hydroxybenzyl glycosides generally. If so, these glycosides represent a new *structural* type of alkali-labile glycoside. The mechanism of their decomposition may be regarded as a variant of the β-elimination which occurs in the glycosides of Ballou's third group. In this interpretation of the "hydroxybenzyl elimination," *o*-hydroxybenzyl derivatives are seen as enols of β-substituted ketones, and *p*-hydroxybenzyl derivatives as vinylogs of such ketones.

Once evidence was obtained concerning the over-all mechanism of the hydrolysis, a study was made to determine the order of ejection of the glucosyloxy and cyano groups from the benzyl carbon of dhurrin. Serial analyses for each of the final products of the reaction were performed during the initial phase of the decomposition at a pH less than 8, where the over-all rate is slow. The results are shown in Figure 3. The appearance of glucose was linear with time, but the rates of release of cyanide and *p*-hydroxybenzaldehyde were initially much lower than that for glucose, and they steadily increased over the period studied. The data are not sufficiently precise to show whether one of the possible pathways A or B was followed to the exclusion of the other, but they do show that the predominant pathway is A, in which the glucosyloxy group is ejected first, generating *p*-hydroxymandelonitrile (**6**) as an intermediate. Since the decomposition of this cyanohydrin gives cyanide and *p*-hydroxybenzaldehyde (**9**) the curves for these two products are essentially identical, and their rate of formation increases as the cyanohydrin accumulates.

(9) M. Somogyi, *J. Biol. Chem.*, **195**, 19 (1952). The "colorimetric" formulation was used.

(10) H. S. Isbell, *Ann. Rev. Biochem.*, **12**, 205 (1943).

(11) C. E. Ballou and K. P. Link, *J. Am. Chem. Soc.*, **71**, 3743 (1949).

(12) W. R. Dunstan and T. A. Henry, *Phil. Trans. Roy. Soc. London, Ser. A*, **199**, 399 (1902).

(13) S. Larsson and B. Lindberg, *Acta Chem. Scand.*, **16**, 1757 (1962).

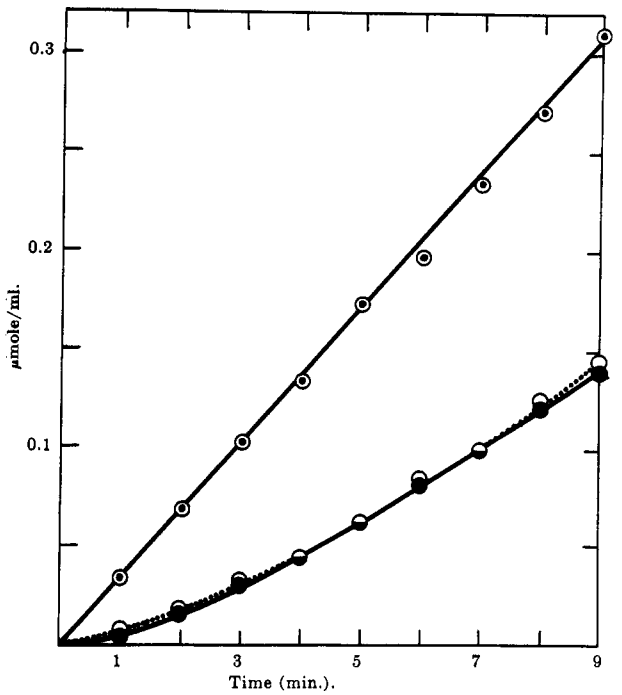


Figure 3.—Initial rates of formation of glucose (⊙), *p*-hydroxybenzaldehyde (○), and cyanide (●) from dhurrin at pH *ca.* 7.7 at 25°.

Our formulation (pathway A) implies that the decomposition of *p*-hydroxymandelonitrile proceeds in the same way as the first step in the decomposition of dhurrin, rather than by the usually accepted mechanism for the decomposition of cyanohydrins, which begins with the removal of a proton from the α-hydroxy group. It seems likely, in the light of the foregoing discussion, that the hydroxybenzyl nature of this cyanohydrin will govern the course of the reaction, but it must be emphasized that the findings relate primarily to the mechanism of the initial cleavage. They provide no real basis for distinguishing between the two possible modes of decomposition of *p*-hydroxymandelonitrile.

Experimental

Materials.—The isolation of dhurrin from *Sorghum vulgare* was described in the previous paper.[1] *p*-Hydroxybenzaldehyde purchased from the Matheson Co. was decolorized and recrystallized from hot water until the melting point was constant. Benzyl β-D-glucopyranoside was prepared as described by Slotta and Heller.[14] *p*-Hydroxybenzyl alcohol and *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald) were purchased from the Aldrich Chemical Co., *o*-hydroxybenzyl alcohol (saligenin) from Eastman Organic Chemicals, Glucostat from the Worthington Biochemical Corp., and sweet almond emulsin from the Nutritional Biochemicals Corp.

Ultraviolet spectra were measured with a Cary Model 11 recording spectrophotometer.

Rate of Hydrolysis of Dhurrin at Various pH Values.—The buffers used, together with the table numbers by which they are listed in the "Biochemists' Handbook"[15] were sodium bicarbonate–carbonate (5M, I = 0.1), sodium borate with added KCl (5Z), and sodium phosphate–borate (5 Universal, universal buffer with citrate and diethylbarbiturate omitted). Dhurrin solution (0.72 mM) and buffer were incubated separately at 25° for 10 min. The reaction was initiated by mixing 1.0 ml. of dhurrin solution and 2.0 ml. of buffer (or, in the experiments to determine the rate constant, 0.10 N sodium hydroxide) in a 1-cm. quartz cuvette. The absorbancy at 330 mμ (*p*-hydroxybenzaldehyde anion) was followed for 10 min. in the Cary Model 11 recording spectrophotometer or in a Beckman Model DU. The cuvette holders were thermostated at 25 ± 0.5°. The slopes of the linear portions (see discussion above) of the curves so obtained were used to compute the rates of formation of *p*-hydroxybenzaldehyde. Since the degree of dissociation of *p*-hydroxybenzaldehyde (pK_a 7.66)[16] changes over the pH range involved, the factor for converting observed absorbancy to total *p*-hydroxybenzaldehyde concentration was determined for each experiment by measuring the absorbancy of a standard solution of *p*-hydroxybenzaldehyde in the buffer used.

Rate of Production of Glucose, Cyanide, and *p*-Hydroxybenzaldehyde at pH 7.7.—After thermal equilibration, 5.6 ml. of 20 mM dhurrin solution and 1.4 ml. of 0.25 M sodium bicarbonate–carbonate buffer, pH 8.2, were mixed. The addition of the dhurrin shifted the pH to about 7.7 (calculated), owing to the low capacity of the buffer in the pH range involved. The mixture was incubated in a water bath at 25 ± 0.2°, and portions were withdrawn at 1-min. intervals; 0.4 ml. of each portion was discharged into Glucostat reagent buffered at pH 6.8 with 0.2 M sodium phosphate, and 0.2 ml. into plain sodium phosphate buffer (1.8 ml.) of the same pH (for cyanide determination). The lowering of the pH quenched the decomposition reaction.

The procedure of Washko and Rice[17] for the determination of glucose with Glucostat reagent (a glucose oxidase–peroxidase–chromogen system) had to be modified because of the strong inhibition of the peroxidase component by the cyanide present in the samples. The difficulty was circumvented by doubling the reagent concentration and using cupric sulfate (0.1 ml. of 0.2 M per tube before addition of the sample) to complex the cyanide.

Cyanide was determined by the modified pyrazolone method described in the previous paper.[1]

In separate experiments, the rates of formation of cyanide and *p*-hydroxybenzaldehyde were compared, by essentially the procedure just described. The samples for *p*-hydroxybenzaldehyde assay were discharged into acid, and the *p*-hydroxybenzaldehyde was measured by the absorption of the undissociated species at 284 mμ.

Methylation of Dhurrin.—Dhurrin (156 mg.) was dissolved in 2 ml. of methanol and treated with an excess of ethereal diazomethane, generated from Diazald. The mixture was kept loosely stoppered at room temperature overnight, after which the solvents were evaporated *in vacuo*. The residue was then dissolved in water and the solution was decolorized with active carbon. A portion of the solution was hydrolyzed with 2 N sulfuric acid at 100° for 2.5 hr., the hydrolysate was neutralized with sodium hydroxide and extracted with ether, and the ether extract was evaporated to dryness. The oily residue gave a crystalline 2,4-dinitrophenylhydrazone melting, after recrystallization from ethanol–ethyl acetate, at 253–254°. This derivative showed no depression in melting point when mixed with an authentic sample of anisaldehyde 2,4-dinitrophenylhydrazone, m.p. 254°. The yield was 103 mg., about 68% of the theoretical from the portion of the crude product taken.

Another portion of the solution was streaked on Whatman No. 3 paper, and the chromatogram was developed with 1-butanol–ethanol–water, 40:11:19. The bands were located by spraying a pilot strip with ammoniacal silver nitrate. The material of the principal band, which had R_f 0.88 and was well separated from dhurrin, was obtained by elution with water. The resulting solution was used for further characterization and for tests of alkali lability.

The formation of an anisaldehyde derivative in high yield is evidence that the major product of the methylation reaction was *p*-methoxymandelonitrile glucoside, and the fact that the ultraviolet spectrum of the purified substance was not altered by alkali confirms this conclusion. Further confirmation was obtained by treating a portion of the solution with emulsin in a Conway cell.[1] The hydrogen cyanide released and the glucose in the residual solution were determined, and the ratio was found to be 1.00:1.03. The identity of the absorption spectrum of the *p*-methoxymandelonitrile glucoside with that of the parent (undissociated) dhurrin was to be expected in view of the fact that the spectra of

(14) K. H. Slotta and H. Heller, *Ber.*, **63**, 1024 (1930).

(15) C. Long, "Biochemists' Handbook," D. Van Nostrand Co., Inc., Princeton, N. J., 1961, pp. 36, 40, and 41.

(16) "The Merck Index," P. G. Stecher, Ed., 7th Ed., Merck and Co., Rahway, N. J., 1960, p. 538.

(17) M. E. Washko and E. W. Rice, *Clin. Chem.*, **7**, 542 (1961).

(18) Ramart-Lucas and Rabaté, *Compt. rend.*, **196**, 1493 (1933).

anisaldehyde and *p*-hydroxybenzaldehyde are essentially identical (Table I), as are those of anisole and phenol.[18]

Enzymatic Synthesis of *p*-Hydroxybenzyl β-D-Glucopyranoside and *o*-Hydroxybenzyl β-D-Glucopyranoside.—The enzymatic synthesis of *o*-hydroxybenzyl β-D-glucoside was reported many years ago by Bourquelot and Hérissey.[19] *p*-Hydroxybenzyl β-D-glucoside has not been synthesized before, but since it is very similar in structure to the *o*-hydroxybenzyl derivative the procedure of Bourquelot and Hérissey was tried. Following their directions, 25 g. of *o*-hydroxybenzyl alcohol, 2 g. of glucose, and 1 g. of emulsin were dissolved in a mixture of 20 ml. of water and 70 ml. of acetone, and the mixture was incubated at 15–20° for 19 days with occasional shaking. The same reaction mixture was used for *p*-hydroxybenzyl glucoside, except that the amount of acetone was increased to 100 ml. because of the lower solubility of *p*-hydroxybenzyl alcohol. When incubation was complete, the mixtures were filtered, and the filtrates were evaporated to sirup in a rotary vacuum evaporator. The sirups were dissolved in 30 ml. of water and extracted with ether to remove the unreacted alcohols. After aeration to remove ether, each solution was incubated with 3 g. of starch-free baker's yeast at 37° until no free glucose could be detected by glucose oxidase paper (Tes-tap, Eli Lilly Co.). The yeast was then filtered off, and the filtrates were decolorized with active carbon.

Portions of the decolorized solutions were streaked on Whatman No. 3 papers, which were then irrigated with 1-butanol–ethanol–water 40:11:19. Two pilot strips were cut from each chromatogram. One strip was sprayed with ammoniacal silver nitrate to detect glucosides and the other with diazotized *p*-nitroaniline–sodium carbonate reagent[20] to detect phenolic compounds. The *o*-hydroxybenzyl alcohol reaction mixture gave five bands positive to ammoniacal silver nitrate, and the *p*-hydroxybenzyl alcohol mixture four, but only one band from each mixture gave color with diazotized *p*-nitroaniline. The bands positive to both spray reagents were cut out and eluted with water, and the concentration of glucoside in the eluates was determined by the anthrone method.[21]

Ramart-Lucas and Rabaté[18] reported that the ultraviolet absorption spectrum of *o*-hydroxybenzyl β-D-glucoside was identical with that of its aglycone, *o*-hydroxybenzyl alcohol. The eluted substances could thus be characterized as the desired hydroxybenzyl glucosides by their ultraviolet spectra, which were examined in both acidic and alkaline solutions. It was found (Table I) that in both cases the spectra were identical, in λ_{max} as well as A_m, with those of the respective hydroxybenzyl alcohols. It was estimated that 0.27 g. of *o*-hydroxybenzyl β-D-glucoside and 0.18 g. of *p*-hydroxybenzyl β-D-glucoside were synthesized in these operations.

Alkaline Methanolysis.—To 4.4 mg. of dhurrin in 10 ml. of dry methanol was added 1.3 ml. of 1.44 *N* barium methoxide. After 2 hr. at room temperature, the reaction was stopped by carbonation with CO_2 gas. Barium carbonate was removed by filtration and the filtrate was concentrated to 3 ml. in a rotary vacuum evaporator. A gummy precipitate formed in the concentrate during a 0.5 hr. standing. This precipitate was filtered off and the clear filtrate was used for paper chromatographic analysis (Whatman No. 1 paper, 1-butanol–ethanol–water 40:11:19, visualization with ammoniacal silver nitrate).

R_f Values.—The R_f values, in 1-butanol–ethanol–water 40:11:19, of the glucosides and sugars separated or identified by paper chromatography in the present work are listed here for convenience.

D-Glucose	0.24
D-Fructose	0.28
Methyl α-D-glucopyranoside	0.37
Dhurrin	0.72
p-Methoxymandelonitrile β-D-glucopyranoside	0.88
o-Hydroxybenzyl β-D-glucopyranoside	0.70
p-Hydroxybenzyl β-D-glucopyranoside	0.62

(19) E. Bourquelot and H. Hérissey, *J. pharm. chim.*, *Ser. 7*, **8**, 49 (1913).
(20) T. Swain, *Biochem. J.*, **53**, 200 (1953).
(21) D. L. Morris, *Science*, **107**, 254 (1948).

The Constituents of *Ecballium elaterium* L. XXI.[1,2] Isomerism in Ring A of the Cucurbitanes

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New evidence shows that the hydrogenation of the diosphenol-containing elatericin B (Ia) proceeds through a 1,4-addition of hydrogen to yield tetrahydroisoelatericin B (3-hydroxy-2-one), whereas the acetoxy derivative (II) proceeds through a 1,2-addition yielding a 2-acetoxy-3-one product (III).

In previous papers dealing with the hydrogenation of the diosphenol-containing cucurbitacins, namely elatericin B (Ia) and elaterin (Ib) it has been reported that the first mole of hydrogen was consumed in the reduction of the double bond on the side chain, whereas the second reduced the enolic double bond of the diosphenol system in ring A.[4] It was expected that products identical with dihydroelatericin A (VII) or dihydrocucurbitacin B (23,24-dihydro VIb) should form. However, different compounds were obtained. The difference was explained in terms of the formation of epimers involving the center of asymmetry at C-2. These conclusions were made on the basis of infrared and ultraviolet spectroscopy as well as optical rotatory dispersion measurements.[4] The n.m.r. spectrum of the hydrogenation product of elatericin B was now found to show a singlet at τ 6.02 and that of its diacetate, a sharp one at τ 5.0. This observation clearly points to the fact that the proton linked to the carbon to which the acetoxy group is also attached has *no neighboring protons* and cannot, therefore, be at the formerly assumed C-2 position. The latter would have displayed of necessity a multiplet due to spin–spin coupling with the adjacent hydrogens at C-1. In order to account for the singlet in the n.m.r. spectrum, the alternate C-3 position for the hydrogen in question had to be considered through a possible 1,4-addition of hydrogen to the diosphenol system, resulting in the conversion of the Δ^1-2-hydroxy-3-keto to a 2-keto-3-hydroxy system which we now call tetrahydroisoelatericin B (IV). In this compound the acetoxy group has probably the most stable α-equatorial orientation.

(1) This investigation was supported by a research grant, CA-2810, from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.
(2) Part XX: D. Lavie and B. S. Benjaminov, *Tetrahedron*, **20**, 2665 (1964).
(3) This author gratefully acknowledges the National Cancer Institute Fellowship (CA-19,319) of the National Institutes of Health, U. S. Public Health Service, and the leave of absence from Rose Polytechnic Institute, Terre Haute, Ind.
(4) D. Lavie, Y. Shvo, O. R. Gottlieb, and E. Glotter, *J. Org. Chem.*, **28**, 1790 (1963), and the references cited therein.