the solvent, some trace impurities may have remained and were responsible for the halogenation reaction, a large quantity (approx. 50 g./liter) of iodine monochloride was added to previously purified *n*-hexane. The mixture was allowed to stand for several days, the remaining iodine monochloride was destroyed with stannous chloride, and the solvent was dried and fractionated. This solvent was used to prepare new solutions of iodine monochloride; however, no change in the behavior of the system was observed. These results indicate that the presence of an impurity is not responsible for the halogenation reaction.

Another portion of purified *n*-hexane was treated with a large amount of iodine monochloride for several days after which excess halogen was destroyed as described above. The resulting solution was concentrated by fractional distillation and an infrared spectrum was obtained of the residue. There was a weak but a definite indication of the carbon-chlorine stretching vibration at 700 cm.$^{-1}$ Unfortunately, this band was largely masked by the carbon-hydrogen rocking vibration at 720 cm.$^{-1}$

A solution of chlorine was prepared in *n*-hexane and the change in the halogen content was followed iodometrically. Although a loss of chlorine was observed, the rate of this loss was much slower than for iodine monochloride. About 50% of chlorine still remained in solution after a 12-hr. period. On the other hand, iodine solutions in this solvent appear to be perfectly stable.

Solutions of iodine monochloride were likewise prepared in purified *n*-pentane, *n*-heptane, isooctane, and cyclohexane. While the first three solvents behaved similarly to the *n*-hexane, it was found that iodine monochloride solutions in cyclohexane appeared to be considerably more stable, although in general one would expect a cyclic hydrocarbon to be more reactive than an aliphatic one.

These results agree with the observations of Buckles and Mills[3] who found it possible to obtain the absorption curve of iodine monochloride in cyclohexane, provided that fresh solutions are used. These authors report an absorption maximum of 466 mμ with a molar absorbancy index of 165.

The experimental evidence obtained in this investigation indicates that iodine monochloride is a very active chlorinating agent for saturated aliphatic hydrocarbons. It seems to react much faster than elemental chlorine and it would consequently seem rather unlikely that the chlorination is preceded by the dissociation of iodine monochloride into molecular iodine and chlorine. It is also interesting to note that iodine is often used as a catalyst in the chlorinations of saturated hydrocarbons. It appears to be quite likely that this catalytic action is due to the intermediate formation of iodine monochloride.

A more detailed investigation of the iodine monochloride reactions with saturated aliphatic hydrocarbons would be of interest. However, such an investigation is not contemplated by the present authors as it is beyond their immediate interests.

(3) R. E. Buckles and J. F. Mills, *J. Am. Chem. Soc.*, **76**, 4845 (1954).

EXPERIMENTAL

Iodine monochloride. Iodine monochloride was prepared by the method of Cornog and Karges.[4] It was first purified by sublimation in a desiccator over phosphorus pentoxide and the obtained product was transferred, in a dry box, to a glass apparatus consisting of a series of bulbs attached to a manifold. The apparatus was evacuated and sealed and the iodine monochloride was fractionally crystallized at least five times. The purified product was transferred to a series of glass bulbs which were sealed until use. The m.p. was 27.2° (lit.[4] 27.2°).

n-Hexane. *n*-Hexane was a Mateson, Coleman, and Bell product and was originally purified by vigorously shaking it with 10% by volume portions of fuming sulfuric acid until the acid layer remained colorless. The solvent was then repeatedly washed with dilute sulfuric acid and with water. This treatment was followed by repeated washing with alkaline permanganate, with water, and drying with barium oxide. The solvent was finally refluxed over phosphorus pentoxide and fractionally distilled through a 1-meter helices-packed column. The purified product had an absorbance of less than 0.01 units, when measured in 5.00-cm. silica cells at 220 mμ, which was superior to the best commercial grade of "research" grade *n*-hexane. The absorption measurements were done on a Cary recording spectrophotometer, Model 11 in the ultraviolet and visible regions of the spectra, and on a Perkin-Elmer infrared spectrometer, Model 13.

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DEPARTMENT OF CHEMISTRY
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

(4) J. Cornog and R. A. Karges, *Inorganic Syntheses*, vol. I, McGraw Hill, New York, N. Y., 1939, p. 165.

Detection of Flavanones by Reduction with Sodium Borohydride[1]

ROBERT M. HOROWITZ

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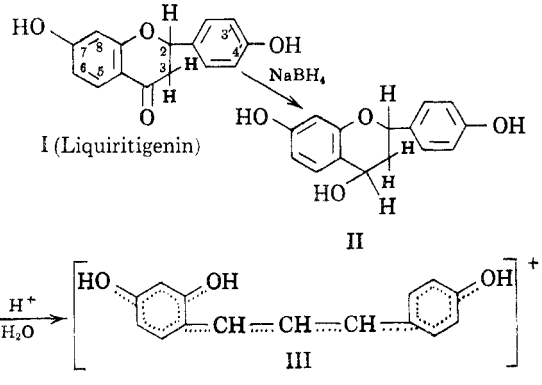
Flavanones may be conveniently reduced under mild conditions by sodium borohydride in aqueous or alcoholic solution. Thus, Pew[2] has reported the

(1) (a) Presented in part before the 131st Meeting of the American Chemical Society, Miami, Fla., April 1957; abstracts p. 58 L. (b) A method which is closely similar has since been reported by E. Eigen, M. Blitz, and E. Gunsberg, *Arch. Biochem. and Biophysics*, **68**, 501 (1957).

(2) J. C. Pew, *J. Am. Chem. Soc.*, **77**, 2831 (1955).

reduction of 6-allyl-8-methoxyflavanone and 6-allyl-3′,4′,8-trimethoxyflavanone to their corresponding 4-hydroxyflavanes by sodium borohydride in alcohol. This reaction may be applied, either in solutions or on paper chromatograms, to the detection of most of the naturally occurring flavanones, since the 4-hydroxyflavanes formed in the reduction give a brilliant purple or blue-red color upon treatment with strong acids. The particular shade of color depends upon the type of hydroxylic substitution, and it is often sufficiently characteristic to be of some use in identification. The following flavanones, most of which are naturally occurring, gave a purple or blue-red color when examined by this method: hesperidin, hesperetin, neohesperidin, naringin, naringenin, isosakuranetin, eriodictyol, homoeriodictyol, butin, liquiritigenin, and liquiritigenin 4′-methyl ether. All of the foregoing compounds have hydroxyl or methoxyl substituents in both the A and B rings. Pinocembrin (5,7-dihydroxyflavanone) and 7-hydroxyflavanone gave a red-orange rather than a purple or blue-red color. 3′,4′-Dihydroxyflavanone and 3′-methoxy-4′-hydroxyflavanone yielded blue colors which were not intense enough to be easily visible on paper chromatograms. It is apparent from this series that the flavanone must have at least one hydroxyl or methoxyl substituent in both the A and B rings in order to yield the maximum development of color.

The probable course of the reaction may be represented as follows:[3]



Because of the close relationship it bears to a flavylium salt, it is certain that an ion of the type III would be deeply colored.

The chalcones, flavones, flavonols, isoflavones, and aurones which were examined were not reduced by the sodium borohydride and failed without exception to give the colors characteristic of the flavanones.[4] Many of them did, however, form bright yellow complexes with the sodium borohydride or its decomposition products and these could be readily observed in ultraviolet light or occasionally in daylight. It is thus possible, by applying this procedure alone, to estimate the number of flavonoids on a chromatogram and to differentiate the flavanones from other types.

Dihydroquercetin (3,3′,4′,5,7-pentahydroxyflavanone), the only 3-hydroxyflavanone which was tested, failed to give a pronounced color either on chromatograms or in solution under the usual conditions for the reaction. When a solution of the substance was boiled with the reducing agent for at least ten minutes before acidifying, a red-orange color was obtained. It has been reported that sodium borohydride reduces dihydroquercetin to a leucoanthocyanidin (conditions not specified) which is converted to cyanidin by air and hydrochloric acid.[5]

As an illustration of the use of this method in the study of plant extracts, a chromatographic examination was made of the products obtained from Douglas fir bark. Dihydroquercetin has been previously reported as the principal flavonoid of this species.[6] Paper chromatograms of an extract of Douglas fir bark, when treated with sodium borohydride and hydrogen chloride, exhibited a small purple spot which had the same R_f value as eriodictyol (3′,4′,5,7-tetrahydroxyflavanone) in 10% acetic acid. In addition, both the substance from Douglas fir bark and eriodictyol reduced ammoniacal silver nitrate, a reaction given by compounds containing an *ortho* dihydroxy group. From this evidence it may be concluded that Douglas fir bark contains as a minor component a flavanone which has been provisionally identified as eriodictyol.[7]

The procedure described here should be of value in detecting the presence of flavanones in plant extracts and should aid in the identification of these compounds from abundant sources such as citrus. It is clear that the method could be adapted to quantitative estimations.

EXPERIMENTAL

A freshly prepared 2% solution of sodium borohydride in methanol was used, although wide variations of concentration are permissible.

Paper chromatograms. The dried chromatogram, hung in a fume hood, is sprayed lightly with the borohydride reagent. After about 1 min. it is placed in a closed container (such as a desiccator) and is fumed with hydrogen chloride gas. (Spraying with aqueous hydrochloric acid is not satisfactory.) A bright, easily visible color develops within a few seconds if the concentration of hydrogen chloride is high and it is usually completely developed in 5–10 minutes. There is a perceptible fading of the color within a few hours.

Solutions. An alcoholic solution (0.1 ml.) of the flavanone or crude extract is treated with an equal volume of the boro-

(3) This scheme is essentially that of T. A. Geissman and R. O. Clinton, *J. Am. Chem. Soc.*, **68**, 700 (1946), for the catalytic reduction of flavanones.

(4) Flavonols and, undoubtedly, other flavonoids can be reduced by lithium aluminum hydride: R. Mirza and R. Robinson, *Nature*, **166**, 997 (1950).

(5) T. Swain, *Chemistry and Industry*, 1144 (1954).

(6) J. C. Pew, *J. Am. Chem. Soc.*, **70**, 3031 (1948).

(7) Pew, (ref. 6) mentions that some samples of Douglas fir wood appear to contain a small amount of naringenin. This would not reduce ammoniacal silver nitrate, however.

hydride reagent. After about 1 min. at room temperature several drops of concentrated hydrochloric or sulfuric acid are added. The color develops at once or after very brief warming. Plant extracts containing leucoanthocyanins give similar colors on addition of hydrochloric acid without the use of sodium borohydride.

Extraction of Douglas fir bark. The powdered bark (50 g.) was extracted in a Soxhlet apparatus first with ether and then with methanol. The methanolic extract was treated with lead acetate; the precipitated lead salts were washed with methanol and decomposed with hydrogen sulfide in methanol. Filtration followed by evaporation of the filtrate yielded a crude crystalline material which was used for paper chromatography.

Chromatography of Douglas fir bark flavonoids. Ascending chromatograms were prepared using 10% acetic acid on Whatman No. 1 paper. After treatment with sodium borohydride and hydrogen chloride three spots were observed: a small purple spot (R_f = 0.29), a large tan spot (R_f = 0.48) (dihydroquercetin) and a bright yellow spot near the origin (R_f = 0.04) (probably quercetin and other flavones). All the spots gave intense reduction of ammoniacal silver nitrate. Eriodictyol had R_f = 0.29 when run simultaneously.

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FRUIT AND VEGETABLE CHEMISTRY LABORATORY
WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
PASADENA, CALIF.

Solvation of Stilbene and Azobenzene Metal Adducts

WALTER BOOS AND GEORGE F WRIGHT

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The addition of alkali metals to unsaturated compounds is facilitated by inclusion of ether in which the adducts are soluble. Little is known about the chemical effects of these ethers. In one instance stable etherates are reported[1] and in another instance an optically active ether has conferred activity to the product from an adduct.[2] Now we have studied the etherates of stilbene and azobenzene metal adducts, which remain after evaporation of dioxahexane solutions.

The sparingly-soluble azobenzene dilithium adduct in 2,5-dioxahexane has been shown to undergo a variety of alkylation reactions.[3] We have confirmed the behavior by preparation from 1-chlorobutane of *N,N'*-di-*n*-butylhydrazobenzene. In order to ascertain the role of the dioxahexane in these metal-adducts systems we have heated the azobenzene-dilithium suspension both at 50° (30 mm.) and also at 100° (5 mm.). In either instance the dry residue has the same yellow color it displays in the suspension. When a weighed amount of this dry diadduct is hydrolyzed by water and then is analyzed for lithium content, the calculated formula weight indicates that 2 molecules of dioxahexane are coordinated with 1 molecule of azobenzene dilithium. Indeed, we have isolated 95% of the 2,5-dioxahexane expected if each lithium atom were coordinated with both oxygens of such a diether. Of interest is the further observation that the azobenzene disodium adduct also is coordinated with 2 molecules of dioxahexane.

If the rate of alkylation of the dimetal adducts is dependent on coordination interchange between the ether and the alkylating agent, then the more reactive stilbene-disodium ought to be less firmly solvated than its azobenzene analogue. But experiment shows that vacuum-evaporation of the stilbene-disodium system at 25° leaves a red residue in which 2 molecules of dioxahexane are included. However, the similarity with the azobenzene disodium adduct no longer prevails when the stilbene-disodium adduct is heated under vacuum at 50° or 100°. Then the ratio is found by titration for alkali to correspond with 2 molecules of adduct per one molecule of dioxahexane.

This evidence that solvation is less firm to the carbon-sodium linkage than it is to the nitrogen-sodium linkage is exemplified further by the observation that the stilbene-disodium containing only one ether-oxygen per two atoms of metal is more active than disodium adduct in which one atom of metal is coordinated with two ether-oxygen atoms. Thus it reacts more rapidly with alkylation agents.

It is of further interest to discover whether the diastereomeric ratio of dialkylation products from stilbene-disodium adducts is altered by the extent of solvation. An alkylation agent must be chosen for which the reaction rate is sufficiently slow that interphase reactivity is minimized in the heterogeneous systems comprising the several solvated species. We have chosen diethyl sulfate which is known to react slowly with the solution of stilbene-disodium adduct in dioxahexane to give 13% of *meso* and 26% of *dd,ll*-3,4-diphenylhexane besides regenerated stilbene.[2]

By contrast the *meso* diastereomer predominates in reactions of the red residues with diethyl sulfate. However, the extent of solvation seems to make little difference in the diastereomeric ratio, although the *meso* to *dd,ll* ratio is slightly higher (35% vs. 14%) from the solvate comprising 1 diadduct per 2 dioxahexane than the ratio (39% vs. 19%) from the 2 diadduct: 1 dioxahexane solvate. While this difference may not be highly significant, it may be

(1) B. M. Mikhailov and N. G. Chernova, *Doklady Akad. Nauk S.S.S.R.*, **74**, 939 (1950), *Chem. Abstr.*, **45**, 4698 (1951).
(2) A. G. Brook, H. L. Cohen, and G. F Wright, *J. Org. Chem.*, **18**, 447 (1953).
(3) J. W. B. Reesor and G. F Wright, *J. Org. Chem.*, **22**, 385 (1957).