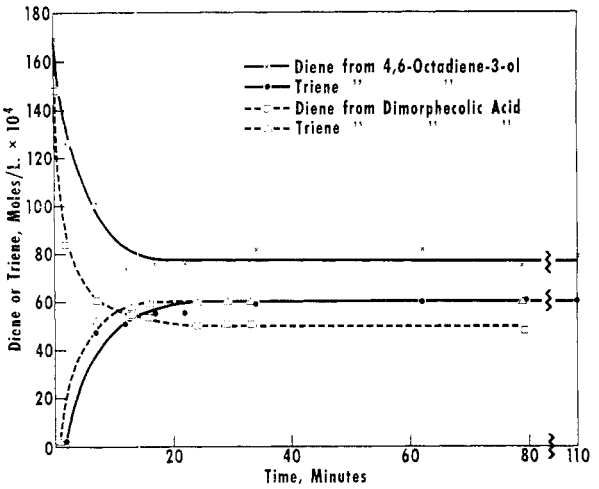

Fig. 1. Effect of hydrogen bromide on 4,6-octadiene-3-ol and on dimorphecolic acid

monoethenoid allylic alcohols with hydrogen bromide, followed by elimination to form triene. Interpretation of the initial substitution as an S_N2 mechanism is favored by the similar uptake of hydrogen bromide in either acetic acid or chloroform solution, by the slower uptake of the more weakly nucleophilic hydrogen chloride, and by the appearance of triene only after the uptake of hydrogen bromide, rather than concurrently as would occur from a carbonium ion intermediate in an S_N1 scheme. However, an S_N1 route is strongly supported by the lack of uptake of hydrogen bromide by the primary alcohol, 2,4-hexadiene-1-ol, which should have a lesser tendency toward carbonium ion formation than the secondary dienols.[11]

EXPERIMENTAL

Dimorphecolic acid. An analytically pure sample was prepared by chromatographing acid isolated by solvent partitioning of mixed acids from *Dimorphotheca aurantiaca* seed oil.[12] A benzene solution of acid (0.5 g.) was added to a silica gel column (5 g.) pretreated with 80% aqueous methanol:hexane (1:1). The pure acid (0.22 g.) was eluted by benzene under nitrogen. It was a semisolid at room temperature. $\lambda_{max}^{C_2H_5OH}$ 231, ϵ 28,800.

Anal. Calcd. for $C_{18}H_{32}O_3$: C, 73.0; H, 10.8. Found: C, 73.3; H, 10.9.

Trans,trans-2,4-hexadiene-1-ol. Ethyl sorbate was reduced by lithium aluminum hydride by a slight modification of the method of Nystrom and Brown.[13] The alcohol was obtained as a colorless mobile liquid. Its 3,5-dinitrobenzoate, prepared according to Reichstein and co-workers,[14] melted at 82–84° (Fisher-Johns[15] block) (lit. m.p., 85°).

Trans,trans-4,6-octadiene-3-ol. Technical grade hexadienal[16] was purified by distillation at 65.5–66°/18 mm., $\lambda_{max}^{C_2H_5OH}$ 271, ϵ 28,700. Hausser, *et al.*[17] reported ϵ 26,500. The distilled hexadienal was condensed with ethylmagnesium bromide according to Kuhn and Grundmann[3] to give the octadienol (78%) $\lambda_{max}^{C_2H_5OH}$ 229, ϵ 24,200. Distillation at 77–79°/20 mm. gave a product having $\lambda_{max}^{C_2H_5OH}$ 229, ϵ 28,400, n_D^{18} 1.4895 (lit., 1.4892).

Hydrogen bromide consumption. Uptake of hydrogen bromide by the unsaturated alcohols was determined by Durbetaki titration[6,7] in benzene–acetic acid solution. For spectral studies, acetic acid only was used as solvent. This solvent change reduced the molar hydrogen bromide uptake to 0.77 (from 0.9 or higher).

Hydrogen bromide reactions. The unsaturated alcohols (0.15–0.2 mmole) were dissolved in 5–10 ml. of glacial acetic acid and were treated with a volume of 0.03–0.05*N* hydrogen bromide in acetic acid found, by prior titration, to be rapidly consumed. At intervals, 0.1-ml. aliquots were removed and diluted to 100 ml. with absolute ethanol. Conjugated diene was determined in 1-cm. cells in a Beckman Model DU spectrophotometer, using the experimentally determined extinction coefficients given above. A molecular extinction of 59,200 at 264 mμ[18] was used for conjugated triene. Data in Fig. 1 are from one of several similar experiments.

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(11) E. R. Alexander, *Principles of Ionic Organic Reactions*, John Wiley & Sons, New York, 1950, p. 41.

(12) C. R. Smith, Jr., M. C. Burnett, T. L. Wilson, R. L. Lohmar, and I. A. Wolff, *J. Am. Oil Chemists' Soc.*, **37**, 320 (1960).

(13) R. F. Nystrom and W. G. Brown, *J. Am. Chem. Soc.*, **69**, 1197, 2548 (1947); *J. Am. Chem. Soc.*, **70**, 3738 (1948).

(14) T. Reichstein, C. Ammann, and G. Trivelli, *Helv. Chim. Acta*, **15**, 261 (1932).

(15) Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

(16) Generously supplied by Union Carbide Chemicals Co.

(17) K. W. Hausser, R. Kuhn, A. Smakula, and M. Hoffer, *Z. physik. Chem.*, **B29**, 371 (1935).

(18) American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed. (1958 revision), Method Cd 7-58.

Specificity of the Phenolic Component for Sakaguchi Reaction[1]

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In 1925, Sakaguchi observed that an intense red color was produced when arginine was treated in alkaline solution with 1-naphthol and hypohalite[2]; it was found later that the reaction was specific for a class of monosubstituted guanidines.[2,3] The specificity of the phenolic component for this reaction has not, however, been studied adequately

(1) (a) This constitutes Paper V in a series *Studies on Sakaguchi Reaction;* for Paper IV, see K. R. Bhattacharya, *Ann. Biochem. Exptl. Med.*, **20**, 57 (1960). (b) Presented in part by K. R. Bhattacharya and J. Datta before the 46th session of the Indian Science Congress Association, Delhi, January 21–28, 1959.

(2) S. Sakaguchi, *J. Biochem. (Tokyo)*, **5**, 25 (1925). See for review R. J. Block and D. Bolling, *The Amino Acid Composition of Proteins and Foods*, 2nd Ed., Charles C. Thomas, Springfield, Ill., 1951, p. 47.

(3) (a) C. J. Weber, *J. Biol. Chem.*, **86**, 217 (1930). (b) J. D. Mold, J. M. Ladino, and E. J. Schantz, *J. Am. Chem Soc.*, **75**, 6321 (1953).

so far, although a few aromatic hydroxy compounds other than 1-naphthol have been employed from time to time in analytical work as useful substitutes for the latter.[4] Color response of various substituted phenols to this reaction was therefore studied in detail. It was hoped that apart from throwing useful light on the mechanism of the reaction, this might also indicate the point of coupling between the guanidine and the phenolic residues in the colored reaction product.[9]

In Table I are listed a few of the results obtained with arginine. That all the indicated positive tests were in response to specific Sakaguchi reactions

TABLE I

COLOR PRODUCTION BY VARIOUS PHENOLIC COMPOUNDS BY SAKAGUCHI REACTION WITH ARGININE

Compound	Color Produced[a]	Relative Intensity of Color[b]
Phenol	LY	++
p-Phenylphenol	—	...
o-Cresol	Y	+++
m-Cresol	GY	++++
p-Cresol	—	...
Thymol	GY	++++
Salicylic acid	Y	++
p-Hydroxybenzoic acid	—	...
o-Aminophenol	LY	±
p-Aminophenol	—	...
Resorcinol	Y	±
Hydroquinone	—	...
o-Chlorophenol	LY	++
p-Chlorophenol	LY	++
2,4-Dichlorophenol	LY	±
2,4,6-Trichlorophenol	—	...
1-Naphthol-8-sulfonic acid	R	++++
1-Naphthol-4-sulfonic acid	—	...
1,4-Naphthoquinone	—	...
5-Quinolinol	RS	++++
2-Methyl-4-quinolinol	—	...
2-Naphthol	B	++
2-Naphthol-6-sulfonic acid	B	+
1-Nitroso-2-naphthol	—	...

[a] Y = Yellow; LY = lemon yellow; GY = golden yellow; R = red; B = brown; RS = reddish saffron;— = none. These refer to the color in alkaline soln. In acid,[10] the color varied from very faint (in the case of a benzene ring) to fairly strong (naphthalene or quinoline ring) yellow. [b] The intensities are based on visual comparison. The larger the number of plus signs, the higher is the color intensity. The sign ± indicates very feeble coloration.

was shown by the fact that no other amino acid nor creatine could replace arginine in these color reactions, but glycocyamine[2,3a] reacted positively like arginine. It is clear from these results that the Sakaguchi reaction is actually a general reaction of phenolic compounds but that a free *para* position in the phenol is specifically required for participation in it. The positive response of a few (though not all) *p*-halophenols (also observed earlier[4]) is admittedly at variance with the latter requirement, but such occasional exceptional behavior of some *p*-halophenols is not without precedent. Thus although the Gibbs indophenol reaction[11a] is in general specific for *para*-unsubstituted phenols, *p*-chlorophenol is able to give the same reaction.[11b] Similarly, in the indophenol reaction between *p*-aminodimethylaniline and phenols in presence of hypohalite, *p*-cresol gives no reaction but *p*-chlorophenol does (although trichlorophenol, as in the present work, does not).[12] This behavior thus appears to be a property inherent in a phenolic *p*-halogen substituent itself which, under the conditions of these reactions, is apparently sufficiently activated to be eliminated or migrated to another position.

The overall nature of the Sakaguchi reaction would thus place it in the general class of the coupling-type reactions of phenols, the point of coupling being limited here to the carbon atom at position *para* with respect to the phenolic hydroxyl.[13] More specifically, however, the great similarity in the circumstances of reaction and in the nature and specificity of the reactants involved (including the singular reactivity of some *p*-halophenols) suggest in particular that there is probably a good deal of similarity between the mechanism of Sakaguchi reaction and those of the two indophenol reactions noted above (and probably other indophenol and indamine reactions as well). This view is further strengthened by our recent finding[9b] that the colored product of this reaction also, like the indophenols, behaves as a typical redox system. Another very similar indophenol reaction is that between ammonia and phenol mediated by hy-

(4) The following compounds have been used: 8-quinolinol,[5] 7-chloro-8-quinolinol, and 5,7-dichloro-8-quinolinol,[5b] 1-naphthol-8-sulfonic acid,[6] 2,4-dichloro-1-naphthol,[7] and 2-naphthol.[8]

(5) (a) S. Sakaguchi, *J. Biochem.* (*Tokyo*), **37**, 231 (1950). (b) J. W. Janus, *Nature*, **177**, 529 (1956).

(6) H. Kraut, E. von Schrader-Beielstein, and M. Weber, *Z. physiol. Chem.*, **286**, 248 (1950). Cited in *Chem. Abstr.*, **47**, 5977-a (1953).

(7) J. McLeish and H. S. A. Sherratt, *Exptl. Cell Research*, **14**, 625 (1958).

(8) P. M. Strocchi and P. Drago, *Ann. chim.* (*Rome*), **44**, 836 (1954). Cited in *Chem. Abstr.*, **49**, 6035-d (1955).

(9) See discussion in (a) K. R. Bhattacharya, J. Datta, and D. K. Roy, *Arch. Biochem. Biophys.*, **77**, 297 (1958); (b) K. R. Bhattacharya, *Nature*, **184**, 53 (1959).

(10) See K. R. Bhattacharya, J. Datta, and D. K. Roy, *Arch. Biochem. Biophys.*, **84**, 377 (1959), for the effect of *p*H on color in the case of 1-naphthol.

(11) (a) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927). See for review F. D. Snell and C. T. Snell, *Colorimetric Methods of Analysis*, Vol. III, 3rd Ed., D. Van Nostrand Company, Inc., New York, N. Y., 1953, pp. 106, 118. (b) M. B. Ettinger and C. C. Ruchhoft, *Anal. Chem.*, **20**, 1191 (1948).

(12) G. U. Houghton and R. G. Pelly, *Analyst*, **62**, 117 (1937).

(13) As neither *para*-substituted phenols nor a simple derivative of 4-quinolinol could participate in the reaction, any linkage through the aromatic hydroxyl group itself[2] or through positions *ortho* or *meta* to it can be safely ruled out. Similarly, the nonparticipation of hydroquinone or 1,4-naphthoquinone would show that the linkage is also not through the 2-position of a preformed *p*-quinone. With 2-naphthol, the linkage is apparently at position 1.

pohalite.[14] The only apparent difference between these two types of reaction is that, whereas in one the phenol is condensed with a simple amine (or ammonia) under the influence of hypohalite,[15] it is condensed with a guanido group in the other. It may thus be speculated that perhaps their products also parallel each other in structure, although it must be remembered that the indophenols and indamines as a class (blue, green or purple) differ appreciably from the products of Sakaguchi reaction (yellow to red) in being more intensely colored.

Incidentally, thymol, 5-quinolinol and 5-chloro-7-iodo-8-quinolinol (the latter in ethyl acetate solution), besides 8-quinolinol, gave sufficiently intense color to be of possible use as substitutes for 1-naphthol in the estimation of arginine by Sakaguchi reaction; 1-naphthol is known to have several disadvantages in this respect.[5b,6,10] With thymol, moreover, blank coloration was practically absent.

EXPERIMENTAL

Arginine and glycocyamine[2,3a] were employed as the guanidine compounds. Because of the advantage of a clear contrast between the color of the spot and that of the surrounding areas, the reactions were carried out on filter paper[9a] rather than in solution. Briefly, spots of arginine (or glycocyamine) were treated first with 2.5% potassium hydroxide in ethanol, then with a 0.1–0.2% solution of the phenol[16] in a suitable solvent, and finally with aqueous hypobromite solution (0.1–2 g. % bromine in 1*N* potassium hydroxide).

Note added in proof: After this note went to the press, a copy of the paper of Kraut *et al.*[6] has been procured. It has been noted that these workers, while selecting a suitable naphtholsulfonic acid for the method, found 1-naphthol-4-sulfonic acid to be ineffective in producing color by this reaction and so concluded that a free 4-position in 1-naphthol was apparently essential for Sakaguchi reaction.

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(14) A. P. Orr, *Biochem. J.*, **18**, 806 (1924); J. A. Russell, *J. Biol. Chem.*, **156**, 457 (1944).

(15) It is noteworthy that the 2,6-dibromoquinonechlorimine of Gibbs is itself prepared by the action of hypochlorite on 2,6-dibromo-*p*-aminophenol.[11a]

(16) We wish to express our appreciation to Prof. B. D. Tilak of the Department of Chemical Technology, University of Bombay, for generous gifts of 1-naphthol-4-sulfonic acid and 2-naphthol-6-sulfonic acid.

Isolation and Characterization of a Phenol Half-Salt

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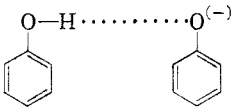
Many observations have been made of anomalous behavior at or near the half neutralization point in the nonaqueous titration of weak acids. In conductometric titration[1] conductivity maxima have frequently been recorded, whereas in potentiometric titration[2] there have been observed corresponding inflections or distortions in the titration curve.

These anomalies have been explained[2a] by the generation, during titration, of an association species in which the generated anion (of either a

R—O—H . . . . . O⁻—R

carboxylic acid or a phenol) protects an equivalent amount of acid, the association being stoichiometric at the half-titration point. This new species is then the acidic participant for the remainder of the titration. The evidence for such a material has been entirely physical and predominately spectroscopic. Pool[3] and Kaufman[4] have presented cryoscopic evidence for the formation of a solid compound composed of one molecule of a base and two molecules of a carboxylic acid, the latter preparing several half-salts between fatty acids and tertiary amines. Analysis of the infrared spectra of dilute solutions of carboxylic acids and tertiary amines[5] has yielded support for this same 2:1 relationship. Recently the alkali half-salts of several carboxylic peptide precursors have been described.[6] In the case of phenols, the evidence for this relationship with bases has been heretofore titrimetric The structural requirements of an unhindered —OH group,[1b,2a,b] and for the exclusion of appreciable amounts of polar solvents (as hydrogen bonding competitors)[1b] imply that the acid-anion structure is a dimer as shown:



We have found that the inclusion of a sterically hindered formamido group *para* to the phenolic —OH group greatly increases the stability of these half-salts, permitting their isolation and manipulation as discrete chemical substances.

When a solution of 4-formamido-3,5-xylenol in methyl isobutyl ketone is titrated with tetrabutylammonium hydroxide in solution in a mixture of methanol and isopropanol, there is obtained a titration curve typical of those described earlier.[2a] In addition, however, there is the generation of a

(1) (a) A. A. Maryott, *Journ. of Res. Nat. Bureau. Standards*, **38**, 527 (1947). (b) D. B. Bruss and G. A. Harlow, *Anal. Chem.*, **30**, 1836 (1958).

(2) (a) G. A. Harlow, and D. B. Bruss, *Anal. Chem.*, **30**, 1833 (1958). (b) H. B. van der Heijde, *Anal. Chem. Acta*, **16**, 392 (1957).

(3) W. O. Pool, H. J. Harwood, and A. W. Ralston, *J. Am. Chem. Soc.*, **67**, 775 (1945).

(4) S. Kaufman, and C. R. Singleterry, *J. Phys. Chem.*, **56**, 604 (1952).

(5) G. M. Barrow, *et al.*, *J. Am. Chem. Soc.*, **76**, 5211 (1954). *J. Am. Chem. Soc.* **77**, 4475 (1955). *J. Am. Chem. Soc.*, **78**, 5802 (1956).

(6) M. Goodman, and K. C. Stueben, *J. Org. Chem.*, **24**, 112 (1959).