product crystallized. Two recrystallizations from ethyl acetate afforded the pure salt, 9.8 g (78%), as prisms: mp 89–91°; ir ($CHCl_3$) 2720, 2470 ($NH_3^+$), 2205 (C≡N), 1610 $cm^{-1}$ ($CO_2$); nmr $\tau$ 8.33 (m, 6, $CH_2$), 7.4 and 7.00 (m, 4, $CH_2$), 6.80 (q, 4, $CH_2N$), 6.04 (q, 4, $CH_2O$), −0.3 (s, 2).

*Anal.* Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10. Found: C, 61.60; H, 7.86; N, 11.28.

**B.**—To a solution of α-cyanohexylideneacetic acid[4] (1.65 g, 0.01 mol) in a minimum amount of hot ethyl acetate was added morpholine (0.9 g, 0.01 mol). On cooling the salt crystallized and had mp 88–91° not depressed in admixture with the material above.

**α-Cyanocyclohexylideneacetic Acid.**—To the salt **4** (2.5 g, 0.01 mol) in 50% aqueous ethanol (7 ml) was added excess concentrated hydrochloric acid. The free acid which precipitated was filtered and recrystallized from water to give 1.4 g (87%) of α-cyanocyclohexylideneacetic acid, mp 108–110°, not depressed in admixture with authentic material[4] of the same melting point.

The following compounds were prepared by similar procedures.

**α-Cyanocyclopentylideneacetic acid morpholinium salt** was obtained in 75% yield after recrystallization from ethyl acetate: mp 106–109° dec; ir ($CHCl_3$) 2740, 2475 ($NH_2^+$), 2220 (C≡N), 1625 $cm^{-1}$ ($CO_2$); nmr $\tau$ 8.22 (m, 4, $CH_2$), 7.13 (m, 4, $CH_2$), 6.78 (q, 4, $CH_2N$), 6.07 (q, 4, $CH_2O$), −0.40 (s, 2).

*Anal.* Calcd for $C_{12}H_{18}N_2O_3$: C, 60.49; H, 7.61; N, 11.76. Found: C, 60.30; H, 7.85; N, 11.75.

**α-Cyanocyclopentylideneacetic Acid.**—The free acid had mp 131–134° ($H_2O$) (lit.[13] mp 130–131°).

**α-Cyanocyclododecylideneacetic acid morpholinium salt** was obtained in 73% yield after recrystallization from ethyl acetate: mp 115–118° dec; ir ($CHCl_3$) 2717, 2470 ($NH_2^+$), 2215 (C≡N), 1615 $cm^{-1}$ ($CO_2$); nmr $\tau$ 8.58 (m, 18, $CH_2$), 7.50 and 7.12 (m, 4, $CH_2$), 6.78 (m, 4, $CH_2N$), 6.06 (m, 4, $CH_2O$), −0.21 (s, 2).

*Anal.* Calcd for $C_{19}H_{32}N_2O_3$: C, 67.82; H, 9.59; N, 8.33. Found: C, 67.80; H, 9.28; N, 8.26.

**α-Cyanocyclododecylideneacetic Acid.**—The free acid had mp 164–167° ($H_2O$ containing a little ethanol); ir (Nujol) 2640 (bonded OH), 2215 (C≡N), 1690 $cm^{-1}$ (C=O); nmr $\tau$ 8.60 (m, 18, $CH_2$), 7.25 (m, 4, $CH_2$), 0.38 (m, 1, acidic H).

*Anal.* Calcd for $C_{15}H_{23}NO_2$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.21; H, 9.28; N, 5.61.

**α-Cyanoisobutylideneacetic acid dimethylammonium salt** was obtained in 63% yield after recrystallization from ethyl acetate: mp 114–116° dec; ir ($CHCl_3$) 2740, 2440 ($NH_2^+$), 2205 (C≡N), 1630 $cm^{-1}$ ($CO_2$); nmr $\tau$ 8.89 [d, 6, $J$ = 7 Hz, $CH(CH_3)_2$], 7.28 [s, 6, N $(CH_3)_2$], 6.98 [m, 1, $CH(CH_3)_2$], 2.68 (d, 1, $J$ = 10 Hz, vinyl H), 0.22 (m, 2).

*Anal.* Calcd for $C_9H_{16}N_2O_2$: C, 58.67; H, 8.75; N, 15.21. Found: C, 58.81; H, 8.66; N, 15.02.

**α-Cyanoisobutylideneacetic acid.**—The free acid had mp 87–89° (chloroform–methylcyclohexane) (lit.[14] mp 89°).

**Attempted Preparation of 6 (R = Cyclohexyl).**—1-Morpholino-1-cyclohexene (1.7 g, 0.01 mol) in benzene or ethyl acetate was treated with cyclohexylcyanoacetic acid[6] (1.7 g, 0.01 mol) at the reflux temperature for 2 hr. Evaporation of the solvent afforded an oil (*ca.* 3.4 g) which partially solidified on standing. Trituration with petroleum ether afforded a solid which on recrystallization from chloroform–petroleum ether gave 1.5 g of a solid, mp 96–98°, which from its nmr spectrum appeared to be the morpholine salt of cyclohexylcyanoacetic acid.

*Anal.* Calcd for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.02. Found: C, 61.84; H, 8.95; N, 10.60.

The compound dissolved in water and acidified with concentrated hydrochloric acid gave cyclohexylcyanoacetic acid, mp and mmp 79–81°. Evaporation of the petroleum ether extracts (above) gave an oil which was shown to be cyclohexanone by its ir spectrum.

**Registry No.**—1 (R = $CH_2CN$), 372-09-8; **4**, 27521-93-3; α-cyanocyclopentylideneacetic acid, 21369-42-6; α-cyanocyclododecylideneacetic acid morpholinium salt, 27521-90-0; α-cyanocyclododecylideneacetic acid, 27521-91-1; α-cyanoisobutylideneacetic acid dimethylammonium salt, 27521-92-2; cyclohexylcyanoacetic acid morpholinium salt, 27521-88-6.

(13) G. A. R. Kon and J. F. Thorpe, *J. Chem. Soc.*, **115**, 686 (1919).
(14) R. A. Letch and R. P. Linstead, *ibid.*, 443 (1932).

## The Bromination of *tert*-Butylbenzene in Trifluoroacetic Acid. The Meta Partial Rate Factor

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The tritium exchange, noncatalytic bromination, and chlorination reactions of toluene, *tert*-butylbenzene, and other alkylbenzenes in trifluoroacetic acid and other mixed solvents rich in trifluoroacetic acid were examined to clarify the role of the solvent, in particular a nonnucleophilic solvent, in the determination of the substituent effects of alkyl groups.[2-6] The order of reactivity for the *p*-alkyl groups depends on the solvent. For the extreme case of the bromination reaction, $k_{p\text{-Me}}/k_{p\text{-}t\text{-Bu}}$ is 3.0 for acetic acid and 0.67 for trifluoroacetic acid.[3,4] The reversal in reactivity may be attributed, largely, to the selective increase in the free energy of solution (activity coefficient) of *tert*-butylbenzene in trifluoroacetic acid.[5] This interpretation is supported by the fact that $o_f^{t\text{-Bu}}$ and $m_f^{t\text{-Bu}}$ are unusually large for tritium exchange ($m_f^{t\text{-Bu}}$ = 32)[2] and chlorination ($m_f^{t\text{-Bu}}$ = 39)[5] in trifluoroacetic acid rich media. The interpretation is also supported by the finding that the partial molal enthalpy of solution of toluene and *tert*-butylbenzene in acetic acid and trifluoroacetic acid differ significantly and suggest that the activity coefficient of *tert*-butylbenzene is selectively enhanced.[5]

Unfortunately, $m_f^{t\text{-Bu}}$ values for the bromination reaction were not determined in the earlier work.[3,4] Study of the available data for the bromination reaction suggested that, if ground-state solvation effects were important, then *m*-bromo-*tert*-butylbenzene would be produced in a measurable amount. Accordingly, we carried out the bromination of *tert*-butylbenzene under the same conditions used in the prior investigations and analyzed the reaction product by capillary vpc. The bromo-*tert*-butylbenzenes were completely resolved on capillary columns with Apiezon L and Carbowax 20M. To test the procedure, we redetermined the isomer distribution for the bromination of *tert*-butylbenzene in 85% acetic acid.[7] The results obtained by vpc were in good agreement with the results obtained earlier by infrared spectroscopy.[7] The products of the bromination of *tert*-butylbenzene in three solvents rich in trifluoroacetic acid were examined. We were unable to detect *o*-bromo-*tert*-butylbenzene in these product mixtures.[8] The meta isomer, on the other hand, was evident in the chromatograms. In addition, the absorption bands for the meta isomer were apparent in the infrared spectrum of a concentrated

(1) National Science Foundation Undergraduate Research Program Participant.
(2) (a) C. Eaborn and R. Taylor, *Chem. Ind. (London)*, 949 (1959); (b) C. Eaborn and R. Taylor, *J. Chem. Soc.*, 247 (1961).
(3) H. C. Brown and R. A. Wirkkala, *J. Amer. Chem. Soc.*, **88**, 1447 (1966).
(4) W. M. Schubert and D. F. Gurka, *ibid.*, **91**, 1443 (1969).
(5) A. Himoe and L. M. Stock, *ibid.*, **91**, 1452 (1969).
(6) The problems involved in the definition of the substituent effects of alkyl groups are reviewed in ref 4 and 5.
(7) H. C. Brown and L. M. Stock, *ibid.*, **81**, 5615 (1959).
(8) The detection limit is estimated to be 0.05%.

solution of the product obtained in a preparative bromination of *tert*-butylbenzene in trifluoroacetic acid. Known mixtures (similar in composition to the product mixtures) of the isomeric bromo-*tert*-butylbenzenes were used to define the vpc response factors. Replicate analyses were obtained on each reaction product. The results are presented in Table I.

TABLE I

ISOMER DISTRIBUTIONS IN THE BROMINATION OF *tert*-BUTYLBENZENE AT 25°

| Solvent,[a] % | Concentration, $M$: $C_6H_5$-$C_4H_9$] | [Na-Br] | [$Br_2$] | Isomer distribution, mol %: Ortho | Meta | Para |
|---|---|---|---|---|---|---|
| | | | Trifluoroacetic Acid | | | |
| 100 | 0.10 | 0.09 | 0.03 | 0.00 | 0.35 ± 0.10 | 99.65 ± 0.10 |
| 100 | 0.10 | 0.09 | 0.03 | 0.00 | 0.36 ± 0.10 | 99.64 ± 0.10 |
| 100 | | | | Av 0.00 | 0.35 ± 0.01 | 99.65 ± 0.01 |
| 93.3 | 0.10 | 0.09 | 0.03 | 0.00 | 0.43 ± 0.10 | 99.57 ± 0.10 |
| 93.3 | 0.10 | 0.09 | 0.03 | 0.00 | 0.37 ± 0.10 | 99.63 ± 0.10 |
| 93.3 | 0.10 | 0.09 | 0.09 | 0.00 | 0.51 ± 0.10 | 99.49 ± 0.10 |
| 93.3 | | | | Av 0.00 | 0.44 ± 0.07 | 99.56 ± 0.07 |
| 78.3 | 0.10 | 0.09 | 0.03 | 0.00 | 0.34 ± 0.10 | 99.66 ± 0.10 |
| 78.3 | 0.05 | 0.045 | 0.015 | 0.00 | 0.34 ± 0.10 | 99.66 ± 0.10 |
| 78.3 | | | | Av 0.00 | 0.34 ± 0.00 | 99.66 ± 0.00 |
| | | | Acetic Acid | | | |
| 85 | 0.51 | 0 | 0.13 | 1.26 ± 0.20 | 1.72 ± 0.20 | 97.02 ± 0.20 |
| 85[b] | 0.51 | 0 | 0.13 | 1.20 | 1.47 | 97.3 |

[a] Weight per cent of acid. [b] Analysis by infrared spectroscopy: H. C. Brown and L. M. Stock, *J. Amer. Chem. Soc.*, **81**, 5615 (1959).

The partial rate factors for the bromination reaction are presented in Table II.

TABLE II

| Solvent[a] | Partial rate factor: Toluene[b] $o_f^{Me}$ | $m_f^{Me}$ | $p_f^{Me}$ | *tert*-Butylbenzene[c] $m_f^{t\text{-Bu}}$ | $m_f^{t\text{-Bu}}$ | $p_f^{t\text{-Bu}}$ |
|---|---|---|---|---|---|---|
| | | | Trifluoroacetic Acid | | | |
| 100 | 1360 | 10[d] | 12,700 | | 34 | 19,200 |
| 93.3 | 4340 | | 42,400 | | 119 | 59,100 |
| 78.3[e] | 2150 | | 19,300 | | 35 | 20,000 |
| | | | Acetic Acid | | | |
| 85.0 | 600 | 5.5 | 2,420 | 5.2 | 7.3 | 805 |

[a] Weight per cent of acid. [b] Factors for trifluoroacetic acid, ref 3 and 4. Factors for acetic acid: H. C. Brown and L. M. Stock, *J. Amer. Chem. Soc.*, **79**, 1421 (1957). [c] Factors for trifluoroacetic acid are based on the rate data of ref 3 and 4 and the isomer distributions shown in Table I. Factors for acetic acid are based on the rate data of ref 7 and the isomer distributions shown in Table I. [d] Determined by additivity method, ref 3. [e] Note ref 9.

The isomer distributions measured in this study establish that $m_f^{t\text{-Bu}}$ is very large for the bromination reaction in the three solvents rich in trifluoroacetic acid. Indeed, $m_f^{t\text{-Bu}}$ for 93.3% acid is the largest value thus far obtained.[9] These results suggest, as discussed previously[5] for the tritium exchange and chlorination reaction, that the reversal in the relative reactivity at the para position of toluene and *tert*-butylbenzene is, in significant part, the consequence of the selective increase in the free energy of *tert*-butylbenzene in the trifluoroacetic acid solvents.

(9) It is pertinent that there is an uncertainty in the rate constant for the bromination of benzene in 78.3% trifluoroacetic acid.[4] However, there is no uncertainty in the rate data for the bromination of the alkylbenzenes in 93.3% acid.[4]

### Experimental Section

*tert*-Butylbenzene (Phillips, research grade) was used without further purification. Trifluoroacetic acid (Matheson Co.) was used with and without fractionation. There were no discernible differences in the isomer distributions. The bromo-*tert*-butylbenzenes were prepared *via* the *tert*-butylation of acetanilide and subsequent deamination.[10] Highly purified samples were employed to standardize the analytical method. The reaction conditions adopted for the prior work[3,4] were used in this study. The products were isolated in the usual way and analyzed most effectively on either Apiezon L or Carbowax 20M columns (50 m) operated at 160° with a 0.5 ml $min^{-1}$ He flow using a Varian Series 1200 chromatograph equipped with a flame ionization detector.

**Registry No.**—*tert*-Butylbenzene, 98-06-6.

(10) T. F. Crimmins, Thesis, Purdue University Library, 1966.

## The Alkaline Decomposition of Organic Disulfides. IV. A Limitation on the Use of Ellman's Reagent, 2,2′-Dinitro-5,5′-dithiodibenzoic Acid

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About ten years ago Ellman[3] described an ingenious procedure for determining quantitatively sulfhydryl content. An excess of the reagent, 2,2′-dinitro-5,5′-dithiodibenzoic acid sodium salt, reacts by thiol–disulfide exchange to release a 2-nitro-5-mercaptobenzoate anion for each sulfhydryl group present. While the disulfide reagent has only a pale yellow color, the 2-nitro-5-mercaptobenzoate anion, like all nitrothiophenolate anions, has a deep color so that measurement of absorbance at 412 nm, as specified by Ellman, when referred to a standard, is a quantitative measure of the sulfhydryl groups originally present.

Ellman's reagent, specifically, is a $10^{-2}$ $M$ solution of the disulfide in phosphate buffer ($\mu$ = 0.1) at pH 7.0. The sample to be analyzed is mixed with phosphate buffer at pH 8.0 before addition of the reagent. The reasons for the choices of pH, though not explicitly stated, are two: the dithiodicarboxylic acid is scarcely soluble in water though its sodium salt is readily so, and the mercaptide ion is much more highly colored than its conjugate acid.

Since the determination of sulfhydryl groups is a frequently employed procedure and Ellman's method is a very attractive one, it has been cited hundreds of times during the last decade. Thus, it is worthwhile to call attention to a hitherto almost unmentioned fact, the extreme sensitivity of Ellman's reagent to alkali, which could lead to erroneous results. Donovan[4] has noted that "Ellman's reagent... showed absorption changes in alkali [concentration not specified] very

(1) Postdoctoral Research Associate, 1969–1970.

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