parently there is no strong driving force for this Grignard to lose the elements of magnesium chloride bromide to form butatriene. The Grignard solution derived from VII or VIII produced a polymeric precipitate on standing. The structure of this polymer will be the subject of a subsequent paper.

Experimental[8]

4-Chloro-2-butyne-1-ol (IV).—To a solution of 860 g. (10 moles) of 2-butyne-1,4-diol (I) in 1 liter of dry benzene and 869 g. (11 moles) of dry pyridine was added dropwise, over a period of 6 hours, 1309 g. (11 moles) of purified thionyl chloride, while the temperature was maintained between 10 and 20°. The reaction mixture was stirred for an additional hour and allowed to stand overnight at room temperature. The mixture was then poured into 2.5 liters of ice-water and the benzene layer was separated. The aqueous layer was extracted with four 1-liter portions of ether and the ether extracts were combined with the original benzene layer. The combined organic extracts were washed with a saturated sodium bicarbonate solution, then with cold water and dried over Drierite. The solvents were removed by flash distillation and the residue was fractionated through an 18-inch Vigreux column to yield 640 g. (61%) of colorless 4-chloro-2-butyne-1-ol (IV), b.p. 50° (0.5 mm.), $n^{25}D$ 1.4980, d^{25}_4 1.2049; and 184 g. (15%) of 1,4-dichloro-2-butyne, b.p. 70° (20 mm.). IV was a powerful skin irritant.

Anal. Calcd. for C_4H_5ClO: C, 49.96; H, 4.82; Cl, 33.92. Found: C, 49.92; H, 4.64; Cl, 33.96.

4-Bromo-2-butyne-1-ol (V).—A solution of 31 g. (0.30 mole) of sodium bromide and 26 g. (0.25 mole) of 4-chloro-2-butyne-1-ol (IV) in 200 ml. of anhydrous methanol was heated under reflux for 12 hours. The cooled reaction mixture was filtered and the filtrate was concentrated to approximately 100 ml. under reduced pressure. The concentrate was diluted with 400 ml. of cold water. The organic layer was dried over Drierite and distilled through a 6-inch, helix-packed column to yield 22.5 g. (62%) of colorless 4-bromo-2-butyne-1-ol (V), b.p. 46° (0.1 mm.), $n^{25}D$ 1.5200, d^{25}_4 1.4742. V also was a strong skin irritant.

Anal. Calcd. for C_4H_5BrO: C, 32.20; H, 3.38; Br, 53.61. Found: C, 32.28; H, 3.41; Br, 53.48.

4-Iodo-2-butyne-1-ol (VI).—To a solution of 165 g. (1.1 moles) of sodium iodide in 600 ml. of anhydrous acetone was added 104.5 g. (1.0 mole) of 4-chloro-2-butyne-1-ol (IV). After the mixture was allowed to stand for 12 hours at room temperature, the sodium chloride was filtered off and the filtrate was concentrated to approximately 200 ml. under reduced pressure. The residue was diluted with 400 ml. of chloroform, and the resulting precipitate was again filtered off. Evaporation of the solvent from the filtrate produced a solid which on recrystallization from a mixture of chloroform and petroleum ether yielded 186 g. (95%) of white leaf-like crystals of 4-iodo-2-butyne-1-ol (VI), m.p. 35.5–36.0°.

Anal. Calcd. for C_4H_5IO: C, 24.50; H, 2.57; I, 64.74. Found: C, 24.35; H, 2.50; I, 64.83.

1-Chloro-4-bromo-2-butyne (VII).—To 209 g. (2.0 moles) of 4-chloro-2-butyne-1-ol (IV) was added dropwise with external cooling over a period of 4 hours, 190 g. (0.68 mole) of freshly distilled phosphorus tribromide in 100 ml. of anhydrous chloroform. After the reaction mixture had been stirred overnight at room temperature, it was diluted with 1 liter of cold water. The chloroform layer was washed with a saturated sodium bicarbonate solution and dried over Drierite. The chloroform was removed by distillation under reduced pressure, and the residue was fractionated through a 12-inch, helix-packed column to yield 235 g. (70%) of 1-chloro-4-bromo-2-butyne (VII), b.p. 45° (2 mm.), $n^{25}D$ 1.5470, d^{25}_4 1.6174. The colorless, unpleasant smelling VII was a powerful skin irritant and a lachrymator.

Anal. Calcd. for C_4H_4BrCl: C, 28.68; H, 2.40; halogen, 68.92. Found: C, 28.72; H, 2.31; halogen, 68.80.

1-Chloro-4-iodo-2-butyne (VIII).—To a solution of 49.0 g. (0.250 mole) of 4-iodo-2-butyne-1-ol (VI) in 150 ml. of anhydrous chloroform was added dropwise with external cooling over a 4-hour period a solution of 11.5 g. (0.084 mole) of freshly distilled phosphorus trichloride in 50 ml. of chloroform. After the reaction mixture was stirred overnight at room temperature, it was diluted with 500 ml. of ice-water. The chloroform layer was separated, washed with a saturated sodium carbonate solution and dried over Drierite. The chloroform was removed under reduced pressure in an atmosphere of oxygen-free nitrogen, and the residue was fractionated through a 6-inch, helix-packed column under a nitrogen atmosphere to yield 20 g. (37%) of pale yellow 1-chloro-4-iodo-2-butyne (VIII), b.p. 61° (0.6 mm.), $n^{25}D$ 1.5920, d^{25}_4 1.9890. VIII, which was a vesicant and a lachrymator, polymerized rapidly on exposure to air. During one distillation, VIII underwent a violent decomposition.

Anal. Calcd. for C_4H_4ClI: C, 22.40; H, 1.88; halogen, 75.72. Found: C, 21.90; H, 1.83; halogen, 75.61.

1-Bromo-4-iodo-2-butyne (IX).—A cooled solution of 49 g. (0.250 mole) of 4-iodo-2-butyne-1-ol (VI) in 150 ml. of anhydrous chloroform was treated under nitrogen with 22.8 g. (0.084 mole) of freshly distilled phosphorus tribromide in 50 ml. of anhydrous chloroform added dropwise over a period of 4 hours. After the reaction mixture was stirred overnight at room temperature, it was diluted with 500 ml. of ice-water. The chloroform layer was extracted with a saturated sodium bicarbonate solution and dried over Drierite. The solvent was removed under reduced pressure, and the residue was recrystallized from a mixture of chloroform and petroleum ether to yield 58 g. (90%) of highly unstable, very pale yellow needles of 1-bromo-4-iodo-2-butyne (IX), m.p. 20–22°.

Anal. Calcd. for C_4H_4BrI: C, 18.55; H, 1.55; halogen, 79.90. Found: C, 18.38; H, 1.51; halogen, 79.98.

Grignard Reagents of Dihalides.—The Grignard reagents were prepared in a 200-ml. creased flask stirred with a 1700-r.p.m. stirring motor. In agreement with Johnson,[5] it was found that 1,4-dichloro-2-butyne (II) would not form a Grignard reagent. Even when II was entrained with methyl iodide, no appreciable reaction took place. However, both 1-chloro-4-bromo-2-butyne (VII) and 1-chloro-4-iodo-2-butyne (VIII) smoothly formed a Grignard reagent. The presence of a true Grignard reagent and not the elimination of both of the halogens was indicated by the vigorous reaction upon the addition of water. Treatment of an aliquot of the Grignard solution with a solution of Michler ketone, followed by a trace of iodine, gave the characteristic color test.[6]

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(8) Analyses were performed by Arthur Tomasewski, Robert Keen and James French. All melting points are corrected.

Partition Chromatographic Separation of Aromatic Acids[1]

BY P. M. BHARGAVA AND CHARLES HEIDELBERGER

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During the course of work on the metabolism of dibenzanthracene-9,10-C^{14} in mice, purification and resolution of mixtures of radioactive organic aromatic acids was sought through a column partition chromatographic procedure. A survey of the literature[2] indicated that little work[3,4] had been done on the separation of aromatic acids (excepting the naturally occurring aromatic amino acids) using this technique. We have found a

(1) This investigation was supported in part by a research grant C-1132 from the National Cancer Institute of the National Institutes of Health, Public Health Service, and in part by a grant from the Wisconsin Section of the American Cancer Society.

(2) See, among others, R. L. M. Synge, *Analyst*, **71**, 256 (1946); F. A. Robinson, *Pharmaceutical J.*, **158**, 46 (1947); T. I. Williams, *Anal. Chim. Acta*, **2**, 635 (1948); H. H. Strain, *Anal. Chem.*, **21**, 75 (1949); **22**, 45 (1950); **23**, 25 (1951); and H. H. Strain, T. R. Sato and J. Engelke, *ibid.*, **26**, 90 (1954).

(3) N. Gordon and M. Beroza, *Anal. Chem.*, **24**, 1968 (1952).

(4) S. Gottlieb, THIS JOURNAL, **70**, 423 (1948).

TABLE I

BEHAVIOR OF SINGLE AROMATIC ACIDS ON A SILICIC ACID COLUMN[a]

Acid	Mg. put on column	State in which applied to the column	Fractions in which eluted[b]	Peak fraction	Recovery, %
5,6,7,8-Tetrahydro-2-naphthoic	2.81	Solution	6–15(10)	8	108
1-Hydroxy-2-naphthoic	3.77	Suspension	8–19(12)	12	97.4
1-Hydroxy-2-naphthoic[c]	31.5	Suspension	6–23(18)	..	...
3,4-Dihydro-2-naphthoic[d]	2.25	Suspension	12–19 (8)	13–14	96
1,2,3,4-Tetrahydro-2-naphthoic[d]	3.50	Solution	13–22(10)	14	99.4
1,4-Dihydro-2-naphthoic[d]	4.21	Suspension	13–26(14)	15	93.3
2-Hydroxy-4-methylbenzoic	3.56	Solution	13–30 (18)	17	97
2-Naphthoic	5.10	Suspension	16–27(12)	19	97.6
2-Naphthoic[e]	1.72	Suspension	18–28(11)	20	93
Diphenylacetic	3.72	Solution	16–27(12)	19	97.6
3-Hydroxy-2-naphthoic[f]	2.50	Solution	19–30(12)	24	80
Benzoic	2.16	Solution	27–36(10)	29	99.6
Benzoic[e]	3.44	Solution	27–36(10)	29	96.5
Benzoic	30.0	Solution	26–48(23)	28	84
Benzoic	100.0	Suspension	22–53(32)	25	84.3
Salicylic	2.82	Solution	26–40(15)	30	92.9
1-Naphthylacetic[g]	4.20	Solution	29–47(19)	34	82.1

[a] A 23 ± 0.5 cm. column was used in all these separations. [b] These include the fractions on either side of the peak tube, in which *any* acid was detectable by titration (*i.e.*, fractions requiring at least 0.005 ml. of alkali in excess of the blank, which required 0.005–0.01 ml.); the total number of fractions in which the acid was eluted, is given in parentheses. [c] The titrated values for the fractions in which the acid was eluted, were irregular and did not follow the chromatographic pattern; the recovery was also very low, suggesting that the quantity was very much beyond the capacity of the column *for this acid.* [d] The gift of these acids from Dr. J. L. Hartwell is gratefully acknowledged. [e] No ethyl acetate was used. [f] Eastman Kodak Co. Technical product was used. [g] M.p. 114–133°, showing that the sample was impure.

mixture of 90% aqueous methanol and 0.5 *N* sulfuric acid (9:1) as the stationary phase on a silicic acid column, and ligroin (Skellysolve B) as the mobile phase (both solvents equilibrated with each other), to be satisfactory for the separation of several aromatic acids. The following solvent systems (mobile phase/stationary phase, each phase equilibrated with the other unless otherwise stated), some of which have been described elsewhere, were tried also but failed to give the desired separation: (a) 35:65 butanol + chloroform/0.5 *N* sulfuric acid[5]; (b) benzene/0.5 *N* sulfuric acid; (c) 1:1 ethyl acetate + chloroform/0.5 *N* sulfuric acid; (d) butanol/ammonium carbonate + ammonium hydroxide; (e) 1:1 ligroin + benzene/90% aqueous methanol; (f) ligroin/0.5 *N* sulfuric acid; (g) ligroin/90% aqueous methanol, both unequilibrated; and (h) ligroin (equilibrated)/9:1 methanol + a formic–acetic acid mixture (unequilibrated).[3] With these systems, the acids that were tried (benzoic, 2-naphthoic, *p*-hydroxybenzoic and 2-phenylphenanthrene-3,2′-dicarboxylic acids) were either eluted with the solvent front (a to e), were not eluted at all (f), or were dispersed too widely in the eluant without any separation and with irreproducible results (g). In case of the last system (h), the acids were eluted along with a very large excess of extraneous acid (presumably acetic or/and formic) making estimation of the organic acids by titration and adaptation to radioactive metabolites impractical.

We have used two different silicic acid column lengths, 23 ± 0.5 cm. and 51 ± 3 cm. The results were found to be reproducible within ± one 2-ml. fraction, as long as the column length was constant within ±0.5 cm. The quantities used were ordinarily of the order of a few milligrams, the recovery being almost quantitative; when a sample of C^{14}-labeled 2-naphthoic acid was run in microgram quantity, the radioactivity followed the titration curve of the acid eluted when milligram quantities were run. It was of interest to note that when certain insoluble acids (or soluble acids in large quantities) were put on the column as a suspension, they were eluted quantitatively in the same chromatographic fashion as smaller quantities put on the column in solution. It is possible that in such cases, the acid eluted in the peak fractions, passes through the column as a supersaturated solution. An increase of the quantity of acid beyond a certain limit (apparently depending on the solubility of the acid in the external phase solvent), and also an increase in the length of the column, caused an increased dispersion of the acid in the eluant, particularly beyond the peak fraction. When a very large excess of the acid was used, it no longer followed a chromatographic pattern (see Table I).

The results obtained are shown in Table I for acids tried singly and in Table II for mixtures. Even though only a few mixtures of acids were tried, it is apparent from Table I that mixtures of acids could be separated on a small (23 cm.) column; a longer column increases the resolution of mixtures of acids which are eluted in about the same fractions on a shorter column (see Table II, footnote *c*). Re-use of the same column gave unpredictable results.

Phenylalanine, terephthalic acid and 2-phenylphenanthrene-3,2′-dicarboxylic acid were not eluted till the 400th fraction on a 23-cm. column, presumably due to their comparative insolubility in the external phase.

Experimental

Solvents.—Redistilled Skellysolve B (500 ml.) was shaken in a separatory funnel with 50 ml. of 9:1 aqueous

(5) V. Zbinovsky and R. H. Burris, *Anal. Chem.*, **26**, 208 (1954).

TABLE II

SEPARATION OF MIXTURES OF AROMATIC ACIDS ON SILICIC ACID COLUMNS

Acids	Mg. put on column	State in which applied to the column	Length of column, cm.	Fractions in which eluted[a]	Peak fraction	Recovery, %
5,6,7,8-Tetrahydro-2-naphthoic,	2.96			6–13(8)	8	102.3
2-naphthoic and	2.87	Suspension	23	14–23(10)	16	102.8
salicylic	3.02			27–41(15)	31	96.2
2-Naphthoic and	2.50	Suspension		37–50(14)	40	96.8
benzoic	3.40		50	57–77(21)	62	95.3
cis,trans-Decahydro-2-naphthoic[b]	3.50			14–17(4)	15	Total:
and *cis,cis*-decahydro-2-naphthoic[b]	3.42	Solution	53.5	18–24(7)	19	53.8
3-Hydroxy-2-naphthoic	..			43–54(12)	46	...
and 2-naphthoic[c]	..	Suspension	48	58–77(20)	66	...

[a] Same as footnote *b* for Table I. [b] W. G. Dauben and E. Hoerger, THIS JOURNAL, **73**, 1505 (1951); the gift of these acids from Dr. Dauben is gratefully acknowledged; the chromatographic positions assigned to these acids are arbitrary. [c] These acids cannot be separated on a 23-cm. column (see Table I); the weight and recovery were not determined in this experiment.

methanol (90%)—0.5 *N* sulfuric acid; the upper layer was used as the mobile phase and the lower layer as the stationary phase.

Silicic Acid.—Mallinckrodt analytical reagent grade, 100-mesh silicic acid was used. Fine particles were removed by repeated (5–6 times) suspensions in an excess of distilled water and decantation after an hour. The jelly-like, fully hydrated, acid was then filtered under suction, dried overnight at 120°, and stored in a desiccator.

Preparation of the Column.—Ten grams of silicic acid was ground thoroughly in a mortar with 7 ml. of the stationary phase, added in 2–3 lots; the acid appeared completely *dry* after this treatment. To this was added 25–30 ml. of the mobile phase and the mixture ground into a *uniform* slurry, which was poured from a 50-ml. beaker into a Pyrex column, 35 cm. (or 75 cm., if the height of the packed column was to be about 50 cm.) long and of 1 cm. internal diameter. The glass column had a small constriction, 5 cm. from the bottom, which supported a closely fitting, detachable sintered-glass plate, kept in position by a thick filter paper disc. It is very important that no air bubbles remain trapped in the slurry inside the column as they were found to cause eventual coagulation of the column. Extensive coagulation (beyond 1–2 cm. at the bottom) during the run was found to affect the chromatographic separation adversely. To avoid this, the silicic acid slurry was poured into the column gently, in small lots, and the column tapped after each addition until air bubbles no longer escaped from the top. Five-lb. pressure was applied to pack the column to the desired length, care being taken that the level of the solvent did not drain below that of the silicic acid at any time.

Application of the Acid and Collection of Fractions.—The organic acid was dissolved in 0.2 ml. of reagent grade ethyl acetate and 2 ml. of the equilibrated ligroin; when large quantities of the acid were used, and in case of some less soluble acids, a suspension was obtained. The solution or suspension of the acid was gently transferred, with a dropper, to the column, in which the solvent had been allowed to run down *almost* to the level of the silicic acid; the solvent was again allowed to drain similarly, with the application of pressure (5 lb.). The above process was repeated three times with the addition of 1 ml. of the mobile phase solvent to the column each time. The silicic acid in the column was finally covered with 3–4 ml. of the ligroin, the column immediately attached to an automatic fraction collector (a model manufactured by Gilson Medical Electronics Co., Madison, Wisconsin, was used) and the chromatogram developed with the mobile phase solvent at a pressure of 5 lb. Two-ml. fractions were collected.

The use of 0.2 ml. of ethyl acetate to dissolve the acid(s) before application to the column, was not found to affect the results significantly (see Table I); its use, however, greatly facilitated solution of the acids in the mobile phase.

Treatment of Fractions.—Two ml. of distilled water, 0.2–0.3 ml. of butanol and one drop of phenol red indicator (*p*H 7.4) were added to all the fractions, which were then titrated with 0.006–0.012 *N* sodium hydroxide, added from a microburet. The two-phase mixture was continously stirred with a stream of carbon dioxide-free air. The end-points were very sharp and could be read accurately to ±0.005 ml. of alkali.

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The Stereochemistry of Some Derivatives of Decalin with Angular Substituents[1]

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Hussey, Liao and Baker[2] recently described the synthesis of a new isomer of 10-methyl-2-decalol (IIIA) by a novel approach to bicyclic compounds with angular methyl groups. The procedure involved catalytic hydrogenation of 10-carbethoxy-$\Delta^{1:9}$-2-octalone (IB) and the transformation of the angular carbethoxy group in the product (IIB) into a methyl group. The authors concluded that the 10-carbethoxy-2-decalone (IIB) and all compounds derived from it, including the alcohol IIIA, had the *cis*-configuration at the ring juncture on the basis of the following evidence.

A series of operations converted the 10-carbethoxy-2-decalone (IIB) to a 9-methyldecalin IVA with a refractive index (n^{25}D 1.4802) similar to that of two samples of 9-methyldecalin to which Hibbit and Linstead[3] and Linstead, Millidge and Walpole[4] had assigned the *cis*-configuration. Moreover, this hydrocarbon was isomerized in the presence of aluminum chloride, as had also been observed by Hibbit and Linstead with their sample,[3] to a product with a lower refractive index (n^{25}D 1.4667). This value was similar to one reported by Ruzicka,

(1) This work was supported by institutional grants to the Detroit Institute of Cancer Research from the American Cancer Society, Inc., The American Cancer Society, Southeastern Michigan Division and The Kresge Foundation.

(2) A. S. Hussey, H. P. Liao and R. H. Baker, THIS JOURNAL, **75**, 4727 (1953).

(3) D. C. Hibbit and R. P. Linstead, *J. Chem. Soc.*, 470 (1936). This sample ($n^{16.1}$D 1.4813) was prepared by the catalytic hydrogenation of a 9-methyloctalin.

(4) R. P. Linstead, A. F. Millidge and A. L. Walpole, *ibid.*, 1140 (1937). In this work the hydrocarbon ($n^{12.5}$D 1.4844) was prepared by a Clemmensen reduction of a pure sample of 9-methyl-2-decalone the *cis*-configuration of which was proven by degradation.