acid and dihydroxymalonic ester. The compounds obtained on ring closure were given the structures corresponding to 6-methyl-2′,1′,2,3-fluorenopyridine-4-carboxylic acid and ethyl fluorenodioxindolecarboxylate, respectively, thus assuming ring closure in the 1-position also.

In this work it has been demonstrated conclusively that the cyclization of diethyl 2-fluorenylaminomethylenemalonate yields a derivative of 11-indeno(2,1-f)quinoline (I).

The study involved first the condensation of diethyl ethoxymethylenemalonate with 2-amino-3-nitrofluorene to produce diethyl 2-(3-nitrofluorenyl)-aminomethylenemalonate (III). Cyclization of III in hot diphenyl ether gave 1-hydroxy-2-carbethoxy-5-nitro-11-indeno(2,1-f)quinoline (IV). Hydrolysis of IV yielded 1-hydroxy-2-carboxy-5-nitro-11-indeno(2,1-f)quinoline (V). This acid was decarboxylated by heating in boiling diphenyl ether, producing 1-hydroxy-5-nitro-11-indeno(2,1-f) quinoline (VI). Reduction of the nitro group of VI to yield 1-hydroxy-5-amino-11-indeno(2,1-f)-quinoline (VII) was followed by deamination to form 1-hydroxy-11-indeno(2,1-f)quinoline (VIII).

Diethyl 2-fluorenylaminomethylenemalonate (IX) was obtained by condensing diethyl ethoxymethylenemalonate with 2-aminofluorene. Cyclization of IX, followed by hydrolysis and decarboxylation, also yielded 1-hydroxy-11-indeno-(2,1-f)quinoline.

Experimental

Diethyl 2-(3-Nitrofluorenyl)-aminomethylenemalonate (III).—2-Amino-3-nitrofluorene[4a,b] (28 g.) was placed in a 500-ml. suction flask with 28 g. of diethyl ethoxymethylenemalonate[5] and 20 ml. of 1,4-dioxane. The flask was heated in an oil-bath at 120–125°, and kept under reduced pressure (20–30 mm.) for 15 hours. The crude product was washed with hot ethyl alcohol, and then recrystallized using charcoal from cellosolve; yield 42 g. (88.3%) of bright yellow needles, m.p. 211–213°.

Anal. Calcd. for $C_{21}H_{20}N_2O_6$: C, 63.62; H, 5.08. Found: C, 63.54. 63.66; H, 5.21, 5.28.

1-Hydroxy-2-carbethoxy-5-nitro-11-indeno(2,1-f)quinoline (IV).—Diethyl 2-(3-nitrofluorenyl)-aminomethylenemalonate (III) (36 g.) was added portionwise to 180 ml. of diphenyl ether previously heated to 240°. After the addition the solution was kept at 245° for 20 minutes. After cooling, an equal volume of petroleum ether was added and the mixture cooled in the refrigerator for two hours. The product was filtered, washed with petroleum ether and recrystallized from cellosolve; yield 31 g. (95%) of brown crystals which did not melt below 355°.

Anal. Calcd. for $C_{19}H_{14}N_2O_6$: C, 65.14; H, 4.00. Found: C, 64.98, 65.07; H, 4.24, 4.19.

1-Hydroxy-5-nitro-11-indeno(2,1-f)quinoline (VI).—1-Hydroxy-2-carbethoxy-5-nitro-11-indeno(2,1-f)quinoline (IV) (19 g.) was added to 1 l. of a 5% alcoholic potassium hydroxide solution and heated under a reflux condenser for four hours. The solution was filtered with suction and the filtrate added to hot water. The hot solution was acidified with dilute hydrochloric acid and a brown precipitate separated out. The resulting suspension was allowed to cool and filtered. The yield of 1-hydroxy-2-carboxy-5-nitro-11-indeno(2,1-f)quinoline (V) was 16 g. (97%). No satisfactory solvent was found for recrystallization, so the acid was not isolated in pure form for analysis.

Sixteen grams of V was added portionwise to 200 ml. of boiling diphenyl ether. The temperature was maintained for 15 minutes. An equal volume of petroleum ether was added and the mixture cooled in the refrigerator for two hours. The product was filtered and washed thoroughly with petroleum ether. The dark brown solid was heated under reflux for one hour in 200 ml. of 95% alcohol and filtered. The alcoholic solution was poured into 250 ml. of water and an orange suspension resulted. The mixture was partially evaporated and allowed to cool. A reddish-brown solid separated which was filtered and dried; yield 7.4 g. (52%). Recrystallization from ethyl alcohol gave light reddish-brown crystals which did not melt below 355°.

Anal. Calcd. for $C_{16}H_{10}N_2O_3$: C, 69.06; H, 3.60. Found: C, 68.84, 68.91; H, 3.74, 3.82.

1-Hydroxy-5-amino-11-indeno(2,1-f)quinoline (VII).—Seven grams of VI was suspended in 300 ml. of 80% ethyl alcohol. A solution of 3 g. of calcium chloride in 5 ml. of water, together with 70 g. of zinc dust was added to the suspension, and the whole was thoroughly mixed. The mixture was heated under reflux for two hours. The sludge of zinc dust and zinc oxide was filtered from the boiling solution and extracted with 20 ml. of boiling 80% alcohol. The combined filtrates were then poured into 600 ml. of water, whereupon a light tan precipitate was obtained. This was filtered with suction and dried. The product was recrystallized from ethyl alcohol; yield 3.3 g. (53%), m.p. 239° dec.

Anal. Calcd. for $C_{16}H_{12}N_2O$: C, 77.42; H, 4.84; N, 11.29. Found: C, 77.27, 77.33; H, 5.03, 5.11; N, 11.06.

1-Hydroxy-11-indeno(2,1-f)quinoline (VIII).—Three grams of VII was placed in a mixture of 3.5 ml. of concentrated hydrochloric acid and 6 ml. of water. The suspension was cooled to approximately 0° and a solution of 0.7 g. of sodium nitrite in 2 ml. of water was added with stirring over 30 minutes. Stirring was continued for five minutes after all of the sodium nitrite solution was added. Then 15 ml. of a cold (0°) 50% hypophosphorous acid solution was added during an interval of about five minutes. Stirring was continued for two hours at 0°, and the mixture set in the refrigerator for 20 hours. A light brown solid was filtered off with suction and recrystallized from ethyl alcohol; yield, 1.1 g. (47%), m.p. 284–286°.

Anal. Calcd. for $C_{16}H_{11}NO$: C, 82.40; H, 4.72; N, 6.00. Found: C, 82.29, 82.34; H, 4.77, 4.91; N, 6.21.

Diethyl 2-fluorenylaminomethylenemalonate (IX).—This compound, melting at 143–145°, was prepared in almost quantitative yields by the procedure outlined for III.

Anal. Calcd. for $C_{21}H_{21}NO_4$: C, 71.79; H, 5.98. Found: C, 71.94, 71.87; H, 6.17, 6.26.

1-Hydroxy-2-carbethoxy-11-indeno(2,1-f)quinoline (X).—X was prepared by ring closure of IX by the method used for IV; yield 53%, m.p. 283–285°.

Anal. Calcd. for $C_{19}H_{15}NO_3$: C, 74.75; H, 4.92. Found: C, 74.73, 74.65; H, 5.21, 5.15.

1-Hydroxy-2-carboxy-11-indeno(2,1-f)quinoline (XI).—Twenty-two grams of X was hydrolyzed as for V to yield 18.4 g. (94.8%) of XI, m.p. 309° dec.

Anal. Calcd. for $C_{17}H_{11}NO_3$: C, 73.65; H, 3.97. Found: C, 73.57, 73.51; H, 4.11, 4.16.

1-Hydroxy-11-indeno(2,1-f)quinoline was also prepared in 62% yields from XI by the procedure used for VI; m.p. 287–288°. A mixed melting point with compound VIII gave no depression.

Anal. Calcd. for $C_{16}H_{11}NO$: C, 82.40; H, 4.72. Found: C, 82.39, 82.26; H, 4.81, 4.87.

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(4) (a) Diels, Schill and Tolson, *Ber.*, **35**, 3284 (1902); (b) Porai-Koshits and Nikiforova, *J. Applied Chem. (U. S. S. R.)*, **13**, 215 (1940); *C. A.*, **35**, 625 (1941).

(5) Fuson, Parham and Reed, *J. Org. Chem.*, **11**, 194 (1946).

The Exchange Reaction between Manganate and Permanganate Ions

BY NORMAN A. BONNER AND HERBERT A. POTRATZ

The exchange between manganate and permanganate ions was first investigated by Libby,[1] who found the rate of exchange to be immeasurably

(1) Libby, THIS JOURNAL, **62**, 1930 (1940).

fast. Separation was carried out by precipitation of barium manganate.

Because of the possibility of separation-induced exchange when precipitation methods are used,[2] Hornig, Zimmerman and Libby[3] reinvestigated the exchange using an extraction method of separation. Their results confirm the earlier work.

Independently, we had been investigating this exchange by use of a different extraction method. Our results confirm and, to some extent, extend the findings of Hornig, Zimmerman and Libby. Our separation procedure involves an essentially complete extraction of permanganate into a chloroform solution of triphenylsulfonium chloride. Manganate remains in the aqueous phase.

In the present study we have tried to establish a lower limit for the exchange rate by using short contact times, low concentrations and low temperatures.

Experimental

Reagents.—Standard analytical grade reagents were used in all cases except for the sodium hydroxide, which was a special grade prepared from sodium. The chloroform and ethylene dichloride were purified immediately before use by successive shakings with several portions of concd. sulfuric acid, aqueous sodium hydroxide, alkaline permanganate and water.

Tracer.—The active manganese (2.59-hour Mn^{56}) was prepared by the Szilard–Chalmers method with potassium permanganate solution. About two liters of nearly saturated potassium permanganate solution was exposed to stray neutrons from the Washington University cyclotron. After the irradiation, the solution was passed twice through a sintered glass funnel. The small amount of manganese dioxide formed during the irradiation remained on the filter and carried about half of the total activity. The manganese dioxide was dissolved in dilute nitric acid to which a little hydrogen peroxide had been added. The resulting solution was evaporated to dryness to remove excess peroxide, and then taken up in a few ml. of water.

To prepare the active manganate, a portion of this solution sufficient to give reasonable counting rates in the final samples was added to 4 *f* NaOH. A known amount of 0.03 *f* $KMnO_4$ solution (a large excess compared to the amount of Mn^{++}) was added to oxidize the active Mn^{++} to $MnO_4^=$. The reduction of the excess permanganate to manganate was then accomplished by boiling the solution, the water acting as reducing agent. Complete reduction usually required about an hour.[4] Finally, the active manganate solution was diluted to the proper volume and filtered to remove the small amount of manganese dioxide usually formed.

Radioactivity Measurements.—In all but the runs at the lowest concentration, the solutions were put in ordinary six inch test-tubes and the gamma radiation counted with an aluminum wall Geiger tube. No corrections were made for slight variations in size and shape of individual test-tubes, or for differences in gamma absorption in water and chloroform.

In the run at lowest concentrations, the manganese was converted to Mn^{++}, aliquots were evaporated on microscope cover glasses, and beta radiation was counted on a thin window Geiger counter.

Analysis.—The manganese in the solutions which had been counted was converted to permanganate by periodate oxidation[5] and the optical density measured at 5080 Å. with a Beckman spectrophotometer.

Exchange Experiments and Separation Procedure.—The general procedure was as follows: An aliquot of the active manganate prepared as described above was diluted with ~2 *f* NaOH. A solution of inactive permanganate in ~2 *f* NaOH was also prepared. The permanganate solution contained twice as much manganese as did the manganate solution. The two solutions were cooled to −1°. The volumes and concentrations were such that when the two solutions were mixed, the final NaOH concentration was 2.0 *f*.

For the actual exchange measurement, the two cooled solutions were mixed in a separatory funnel (not precooled), and shaken for about 10 seconds to ensure complete mixing. An equal volume of cooled (−1°) chloroform (or ethylene dichloride) solution containing about 5 moles of triphenylsulfonium chloride per mole of permanganate in the exchange mixture was added as rapidly as possible, and the mixture was shaken for about 30 seconds. The two phases were allowed to separate, one-third of the bottom layer was drawn off and saved, the middle portion was discarded, and the last one-third of the top layer drawn off and saved. The interval from the time of addition of the organic phase until the middle portion was drawn off was about 1 minute. The portions saved were then counted and analyzed as described previously.

For the three runs summarized in Table I, the amounts of manganate and permanganate used were the same in each case, but the volumes of the aqueous solutions were 300, 150, and 30 ml., respectively, for runs 1, 2 and 3.

Results.—In all experiments the ratio of permanganate to manganate concentrations was 2:1 at the time of mixing. At the end of the experiment, the concentrations were about equal, due to reduction of the permanganate by water and traces of impurities. No apparent exchange was caused by this reduction since the tracer was originally in the form of manganate. In all cases the NaOH concentration was 2.0 *f*.

TABLE I

RESULTS OF EXCHANGE EXPERIMENTS

NaOH concentration, 2 *f*; temperature, ~0°C.; contact time, ~15 sec.

Expt. No.	Initial concentration, *f* (MnO_4^-)	Initial concentration, *f* ($MnO_4^=$)	Exchange measured, %	$T_{1/2}$
1	6×10^{-5}	3×10^{-5}	95	<5 s
2	1.2×10^{-4}	0.6×10^{-4}	93	<5 s
3	6×10^{-4}	3×10^{-4}	>88	<5 s

Table I summarizes the results obtained in our three most significant experiments. In 2 and 3 ethylene dichloride was used instead of chloroform. Other experiments at room temperature and/or for longer times indicated complete exchange. It is significant that the fraction exchange is essentially the same in experiments 1 and 2, indicating that the deviation from 100% exchange is due to experimental error. If the value 93% obtained in experiment 2 were accurate, and if the reaction is bimolecular, the per cent. exchange in experiment 1 should be ~75% instead of 95% as observed.

To test for the amount of exchange occurring during extraction, experiments similar to 1 were carried out in which a solution of triphenylsulfonium permanganate in chloroform (or ethylene dichloride) was shaken with an aqueous solution containing active manganate. For 30 seconds

(2) Prestwood and Wahl, THIS JOURNAL, **71**, 3137 (1949).

(3) Hornig, Zimmerman and Libby, *ibid.*, **72**, 3808 (1950).

(4) In some preliminary experiments Mn^{++} was added to the irradiated permanganate before filtration. The oxidation of the active Mn^{++} (prepared from manganese dioxide as described above) to manganate was then attempted by the addition of an equivalent amount of permanganate at room temperature. The resulting manganate solution was filtered repeatedly. When this "solution" was boiled for a few minutes and again filtered, manganese dioxide, which had apparently been in a colloidal state, coagulated and collected on the filter. This precipitate was found to contain most of the activity, while the manganate filtrate was essentially inactive. It thus appears that under the conditions of this experiment (about 4 *f* NaOH) manganate ion and manganese dioxide exchange very slowly, if at all.

(5) Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., Inc., New York, N. Y., 1943, p. 713.

shaking at 0°, ~30% exchange was observed with chloroform and ~5% with ethylene dichloride. Ethylene dichloride could not be used in the experiments at the lower concentrations because the separation of the two phases became very slow with the large volumes used.

The rate constant calculated from experiment 1,[6] assuming the reaction to be bimolecular, is >1500 liter·mole^{-1}·sec.$^{-1}$ at ~0°.

The above results indicate that the exchange is extremely fast even under conditions of low concentrations and low temperature. Admittedly, we have no proof that exchange is not induced by the separation procedure, but considering the nature of the separation process, induced exchange seems unlikely.

(6) Friedlander and Kennedy, "Introduction to Radiochemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 287.

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Density Gradient Centrifugation: A New Separation Technique[1]

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Density gradient centrifugation is a technique for more efficient centrifugal separations of suspended particles. The author is unaware of any previous publication of the procedure outlined below.

A thin layer of the suspension to be fractionated is floated on a solution having a density gradient. If the suspended particles sediment ideally, *i.e.*, as discrete entities, during subsequent centrifugation, those of each sedimentation rate will travel down the tube as a separate zone. Centrifugation may be continued until the particles reach density equilibria, yielding a separation dependent on particle density only. If centrifugation is stopped earlier, a separation dependent on the sedimentation rates of the particles may be obtained. Theoretically, the latter variation should have the higher resolving power since for two particles to have the same sedimentation rates throughout a tube with a density gradient, their densities and ratios of mass to frictional constant must be identical. In addition to the density gradient, aqueous systems will usually have an osmotic pressure gradient. Then for two particles to sediment at the same rate throughout the tube they must not only fulfill the above criteria, but must also change hydration at the same rate with changing osmotic pressure.

This basic procedure can be modified for application to many different problems involving particles and large molecules of either biological or non-biological origin. Appropriate density gradients must be prepared for each situation. The method might be used as a separation procedure in either of the two ways noted above, as a criterion of purity, or as a technique for measuring densities of particles or large molecules. The use of density gradient tubes for density measurements of macroscopic droplets and particles was originated by Linderstrom-Lang.[2,3] In the present case the interpretation of the densities obtained would be complicated by the possible change in the solvation of the particle as it travels down the tube, since, in general, the liquids used for the density gradients in centrifugation are solvents for the particles concerned.

In the exploratory experiments in this Laboratory with density gradient centrifugation two types of non-ideal sedimentation have been recognized. The first type is due to aggregation of particles such as may be observed in any centrifugation. The second type is due to the sedimentation of droplets of solution containing particles. This phenomenon may be demonstrated by centrifuging a layer of a suspension floated on a denser liquid, which, however, has no density gradient. It probably occurs to a limited extent when the density gradient is not steep enough, causing a spreading of the zones.

That particles will sediment as discrete entities in a density gradient tube was shown by experiments with potato yellow-dwarf virus. Half a ml. of virus concentrate prepared by two cycles of differential centrifugation[4] was placed in a 15-ml. centrifuge tube on a density gradient prepared by layering 2 ml. each of sucrose solutions having densities 1.04, 1.08, 1.12, 1.16 and 1.20 in the tube and allowing diffusion to occur for a few days at 0°. After centrifuging at 3200 r.p.m. for 5 hr. in a horizontal head of an International 1-SB centrifuge at 10°, a zone, suspected of being the virus (S^{w}_{20} = 1150s),[4] was visible from 0.9 to 1.4 cm. below the meniscus. Samples were removed from the bottom of this and other desired zones by means of an inverted tip glass capillary and 0.1 *M* neutral phosphate buffer was added to give suitable equivalent dilutions. Their infectivity was compared by inoculating opposite half-leaves of *Nicotiana rustica* L. One-ml. samples which removed the zones 0.9 to 1.4, 1.9 to 2.4, 2.9 to 3.4, and 3.9 to 4.4 cm. from the original position of the meniscus gave a total on 3 half-leaves of 1871, 34, 6 and 1 local lesions, respectively. A second experiment gave similar results.

Even when density gradient centrifugation is performed in an angle head a definite virus zone may be obtained (Fig. 1). The stabilizing effect of sucrose density gradients on boundaries during angle centrifugation[5] may account for the relative sharpness of the zone obtained.

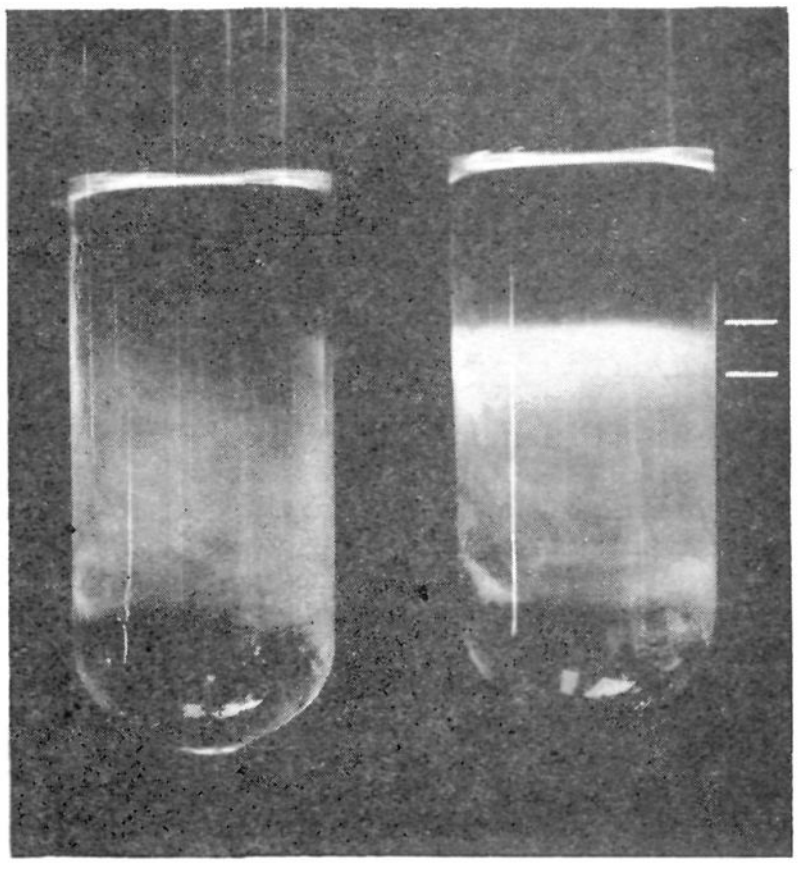

Fig. 1.—Density gradient centrifugation of potato yellow-dwarf virus. The tube on the right contains a concentrate prepared by two cycles of differential centrifugation and one density gradient centrifugation cycle from diseased *N. rustica* plants (virus zone indicated by lines). The tube on the left contains a similar concentrate from normal plants. Three ml. of the concentrate was centrifuged for 20 min. at 10,000 r.p.m. at 5° in a Servall SS-2 through a density gradient prepared in a 50-ml. tube from 7 ml. each of sucrose solutions of densities 1.04, 1.08, 1.12, and 1.16.

(1) This work was supported, in part, by a grant in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) K. Linderstrom-Lang, *Nature*, **139**, 713 (1937).

(3) K. Linderstrom-Lang and H. Lanz, Jr., *Compt. rend. Lab. Carlsberg. Chim. S.*, **21**, 315 (1938).

(4) M. K. Brakke, L. M. Black and R. W. G. Wyckoff, *Am. Jour. Bot.*, in press.

(5) E. G. Pickels, *J. Gen. Physiol.*, **26**, 341 (1942).