TABLE I

EXCHANGE RESULTS

Compound	Percentage exchange in 20 min.	40 min.	60 min.	Half time (min.)	Rate
Nickel(II) orthophosphate[a]	45	67	84	23	2.3×10^{-6}
Nickel(II) pyrophosphate[b]	32	62	81	25	2.2×10^{-6}
Nickel(II) sulfide[c]	34	67	79	26	2.1×10^{-6}
Nickel(II) hydroxide[d]	33	50	70	41	1.3×10^{-6}
Nickel(II) hexacyanoironate(II)[e]	16	37	55	53	1.0×10^{-6}
Tetrapyridine nickel(II) thiocyanate[f]	10	23	35	99	6.1×10^{-7}
Bis-diphenylthiocarbazide nickel(II)[g]	10	19	27	139	4.4×10^{-7}
Bis-salicylaldoxime nickel(II)[h]	5	17	20	173	3.5×10^{-7}
Bis-dimethylglyoxime nickel(II)[i]	No exchange				

[a] R. Tupputi, *Ann. chim. phys.*, [2] **78**, 133 (1840); J. W. Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. XV, Longmans, Green and Co., New York, N. Y., 1936, p. 494. [b] A. Schwarzenberg, *Ann.*, 65, 158 (1848). [c] N. V. Sidgwick, "Chemical Elements and Their Compounds," Vol. II, Oxford University Press, New York, N. Y., 1950, p. 1432. [d] R. Tupputi, ref. *a*, p. 383. [e] P. Walden, *Z. physik. Chem.*, **10**, 710 (1892). [f] G. Spacu and J. Dick, *Z. anal. Chem.*, **71**, 442 (1927). [g] W. Parri, *Giorn. farm. chim.*, **73**, 207 (1924); *C. A.*, **19**, 223 (1925). [h] H. L. Riley, *J. Chem. Soc.*, 895 (1933). [i] L. Tschugaeff, *Compt. rend.*, **145**, 679 (1907).

Radioactive Nickel-63.—The radioactive nickel tracer employed was nickel-63, which emits a 0.057-Mev. beta particle with a half-life of 85 years.[2] This was obtained from the Oak Ridge National Laboratory as nickel(II) chloride in dilute hydrochloric acid.

Exchange Procedure.—All exchange reactions were carried out at 25°. Each reaction mixture consisted of 5.00 ml. of 0.0309 *M* radioactive nickel(II) chloride solution, 10.00 ml. of water and the insoluble nickel(II) compound under consideration. These were prepared by precipitating the nickel(II) ion from 4.05 ml. of 0.0381 *M* nickel(II) chloride solution. This gave equimolar quantities of nickel in the two species involved in each reaction. Reaction mixtures of each compound were vigorously stirred for periods of 20, 40 and 60 minutes. The precipitates were separated by suction filtration and the filtrates were treated with ammoniacal dimethylglyoxime solution to precipitate the nickel as bis-dimethylglyoxime nickel(II). These precipitates were mounted on fritted glass filter discs and the activity of each was measured. All samples were greater than infinite thickness for self absorption. A standard counting sample was prepared from the original radioactive solution.

Radioactivity Apparatus.—A Tracerlab SC-16 Windowless Flow Counter operating in the Geiger region was used in conjunction with a Tracerlab SC-2A Scaler to measure the activities of the samples. All samples were counted for a sufficiently long time to give a standard deviation equal to or less than 1%.

Results

The experimental results are shown in Table I. All systems except the bisdimethylglyoxime nickel(II) one showed exchange for the period of time of the reactions. Plots of the logarithm of one minus fraction exchange against time gave straight lines for all other exchange reactions. The rates of exchange were then calculated from the expression as given by Wahl and Bonner.[3] This equation is said to apply to heterogeneous reactions only if the mixing of isotopes in each phase is rapid as compared to the actual exchange process or to the diffusion across the interface.

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(2) "Isotopes," United States Atomic Energy Commission, Isotopes Division, March, 1951, p. 5.

(3) A. C. Wahl and N. A. Bonner, ref. 1, p. 34.

Dealkylation of Dialkylhydroquinone Diacetates with Aluminum Chloride[1]

BY JACK L. R. WILLIAMS

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When hydroquinone diacetate is submitted to the Fries rearrangement, the product is acetylhydroquinone.[2] However, when 2,5-dimethylphenyl acetate is treated under similar conditions, 2-acetyl-4,6-dimethylphenol results.[3] The acetyl group displaces an ortho-methyl group, rather than taking the open para-position.

In the course of studying the behavior of alkylhydroquinone diacetates, it has been found that dealkylation took place in the case of 2,5-di-*t*-butylhydroquinone and 2,5-di-*t*-amylhydroquinone diacetates. The reactions were carried out using anhydrous aluminum chloride in the absence of a solvent; the products were acetylhydroquinone, instead of the expected dialkylacetohydroquinone, and much tar. The alkyl side chains were probably eliminated as the aluminum chloride complexes which would be expected to polymerize under the reaction conditions.

Experimental

2,5-Di-*t*-butylhydroquinone Diacetate.—To a mixture of 22.2 g. (0.1 mole) of 2,5-di-*t*-butylhydroquinone and 51.0 g. (0.5 mole) of acetic anhydride there was added, with shaking, 5 cc. of a solution of 5 drops of concentrated sulfuric acid in 10 cc. of acetic anhydride. The temperature rose immediately and the reaction mixture was allowed to stand for 1.0 hour, after which time it was poured into 1.0 liter of ice-water. The white crystals were filtered and dried to yield 28.8 g. (94%), m.p. 172–173°. A small sample was recrystallized from benzene and dried *in vacuo*, m.p. 173–174°.

Anal. Calcd. for $C_{18}H_{26}O_4$: C, 70.6; H, 8.6. Found: C, 71.1; H, 8.9.

2,5-Di-*t*-amylhydroquinone Diacetate.—From 25.0 (0.1 mole) of 2,5-di-*t*-amylhydroquinone and 51.0 g. (0.5 mole) of acetic anhydride there was obtained, by the above procedure, 33.0 g. (98%) of 2,5-di-*t*-amylhydroquinone diacetate, m.p. 114–115° (softened at 112–113°).

Anal. Calcd. for $C_{20}H_{30}O_4$: C, 71.8; H, 9.0. Found: C, 72.2; H, 9.4.

Rearrangement of 2,5-Di-*t*-butylhydroquinone Diacetate with Aluminum Chloride.—A mixture of 50 g. (0.16 mole)

(1) Communication No. 1501 from the Kodak Research Laboratories.

(2) G. C. Amin and N. M. Shah, *Org. Syntheses*, **28**, 42 (1948).

(3) K. von Auwers, H. Bundesmann and F. Wieners, *Ann.*, **447**, 162 (1926).

of 2,5-di-*t*-butylhydroquinone diacetate and 109 g. (0.81 mole) of anhydrous aluminum chloride was divided into two portions. One portion was placed in a 1-liter beaker surrounded by an oil-bath maintained at 125–130°. The temperature of the periodically stirred mixture rose to 110° during 20 minutes, after which time the second portion was added, with stirring. After the addition was completed, the reaction mixture was heated at 115–120° (oil-bath 130°) for 0.75 hour. The reaction mixture was cooled rapidly by pouring it into a large enamel tray. The solid reaction residue was stirred with 400 cc. of concentrated hydrochloric acid and 1.0 liter of crushed ice. The ice–acid slurry was heated on the steam-cone until all of the lumps had melted to an oil, after which time the oil was solidified and removed by chilling the mixture in ice. The oil was stirred with 200 cc. of 20% sodium hydroxide. Acidification of the filtered alkaline solution yielded 4.8 g. of yellow solid, m.p. 195°, which, after recrystallization from 25 cc. of ethanol, gave 2.3 g. of pure acetylhydroquinone, m.p. 204–205°. The mixed melting point with an authentic sample of acetylhydroquinone was 204–205°. An additional 0.7 g. of crude acetylhydroquinone, m.p. 201–202°, was obtained from the aqueous mother liquors by further chilling the mixture to 0°. The total yield of acetylhydroquinone, m.p. 201–205°, was 3 g. (12.1%). All attempts to isolate other definite compounds from the tarry reaction product were unsuccessful.

Rearrangement of 2,5-Di-*t*-amylhydroquinone Diacetate with Aluminum Chloride.—When a mixture of 54.5 g. (0.16 mole) of 2,5-di-*t*-amylhydroquinone diacetate and 109 g. (0.81 mole) of anhydrous aluminum chloride was treated as described above for 2,5-di-*t*-butylhydroquinone diacetate, 3.3 g. of crude acetylhydroquinone, m.p. 190–192°, was obtained. Recrystallization gave 1.4 g., m.p. 202.5–203°, yield 5.5%.

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Coconut Milk Factor: The Growth-promoting Substances in Coconut Milk[1]

BY E. M. SHANTZ AND F. C. STEWARD

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Coconut milk is the fluid endosperm that nourishes an immature embryo which later produces a spongy mass of cotyledonary tissue that eventually fills the central cavity of the seed. The dramatic ability of this fluid to foster rapid and random division of otherwise mature cells of higher plants has recently attracted attention. This communication will show that the growth-promoting qualities of coconut milk are due to a number of growth substances. At least three of these substances can be recognized as chemical entities. They have been isolated in small amounts, in crystalline form, and their general characteristics can be described. However, the actual number of such substances in coconut milk that give a distinct growth response when added separately and in low concentration to the nutrient medium may well be considerably larger.

When whole coconut milk is added to a basal medium containing mineral salts, sugar and vitamins it causes a striking increase in the growth by cell division of explants of certain tissues, notably carrot root phloem.[2] This response, under suitably controlled conditions, furnishes an assay method for the active substances[3] Whole coconut milk produces an optimum growth response when added to the culture medium at a level of about 15% by volume, which represents a concentration of about 10,000 p.p.m. on a dry weight basis.

The initial enrichment of the activity was made by treating whole coconut milk with an excess of mercuric acetate after dilution by an equal volume of ethanol. After filtration, the precipitate was suspended in water, treated with H_2S, and filtered to remove the precipitated sulfide. The filtrate was concentrated to a heavy sludge under reduced pressure and the sludge was twice extracted by agitation with 90% ethanol. Removal of the solvent left a dark heavy sirup equivalent to approximately 0.6% of the initial dry material of the coconut milk. This sirup showed optimum activity in the tissue culture growth test when added to the basal medium at a level of about 200 p.p.m.

The above extract was fractionated further by differential solubility in various solvents and by partition chromatography on cellulose. At this stage, fractions were obtained which, although active at much lower concentrations, failed to produce at any concentration a total response which approached that given by the addition of whole coconut milk. The full response, however, could be restored by recombination with certain other fractions which were known to contain, among other things, the bulk of the free amino acids present in the crude extract. A similar effect could be obtained by adding an enzymatic hydrolysate of casein to the basal medium at a level of 500 p.p.m., and to a somewhat lesser extent by the addition of pure amino acid mixtures. The addition of casein hydrolysate alone to the basal medium has but a relatively slight effect upon growth; a pronounced response is obtained only in combination with certain fractions from coconut milk. Extensive work has shown, however, that the degree of dependence upon casein hydrolysate varies somewhat among individual carrot roots from the same stock and to a slightly greater extent among roots from different stocks.

Following the discovery of this effect of added casein hydrolysate, various fractions of the coconut milk concentrate were re-examined to determine which ones, ineffective in themselves, became active when tested in the presence of casein hydrolysate. This was done by measuring the growth of aseptic carrot tissue explants in an otherwise synthetic medium. From fractions which proved to be active in this assay procedure small amounts of three substances have now been isolated which, when tested in the presence of casein hydrolysate, induce a rate of growth which approaches that obtained by the use of whole coconut milk.

Compound A.—This was obtained directly upon evaporation of the alcohol extract described above. The alcohol-soluble portion of the mercury-freed precipitate from 800 gallons of coconut milk was reduced to three liters of aqueous solution. This was filtered to remove a small amount of insoluble residue. Upon drying the filter paper a number of small white crystals could be seen and these were mechanically separated from extraneous material. The 78 mg. of crude crystals thus obtained were twice recrystallized from 2 ml. of hot absolute ethanol, giving a final yield of 56 mg. of fine white needles melting at 240.5° (uncor.). The maximum solubility of this compound in water at room temperature was approximately 40 mg./l. The ultraviolet absorption curve in absolute ethanol is shown in Fig. 1 and the infrared absorption curve in a Nujol mull is shown in Fig. 2. This material gave no color reaction with ninhydrin. *Anal.* C, 75.22; H, 6.93; N, 14.19. The growth response in the carrot tissue bioassay test is shown in Fig. 6.

Further Fractionation Procedure.—An amount of the crude concentrate equivalent to approximately 200 gallons of the original coconut milk was further enriched by several solvent fractionation procedures to yield 2.8 g. of material active in the growth assay at 20 p.p.m. This concentrate was chromatographed in *n*-butanol–acetic acid–water mixture on a column containing 800 g. of finely powdered cellulose and was divided into 300 fractions of 25 ml. each. Each fraction was examined for its ultraviolet absorption, fluorescence under ultraviolet, and intensity of its reaction, if any, with ninhydrin.

(1) This work commenced at the University of Rochester and has been continued at Cornell University. It has been supported by grants to one of us (F. C. S.) from the National Institute of Health. Access to the large bulk of coconut milk was made possible through the generous help of the Grasselli Chemicals Division of the du Pont Co. Mrs. Alice Peabody assisted with the growth assays by tissue culture methods.

(2) S. M Caplin and F. C. Steward, *Science* **108**, 655 (1948).

(3) (a) S. M. Caplin and F. C. Steward, *Nature*, **163**, 920 (1949); (b) F. C. Steward, S. M. Caplin and F. K. Millar, *Ann. Botany*, **16**, 57 (1952).