corresponds closely to the formula $(C_6H_5)_2C(OLi)_2\cdot(C_2H_5)_2O$. However, later experiments in which excess lithium benzoate was used, as well as experiments in which equivalent quantities were employed, gave adducts in which the lithium content varied from 3.8 to 5.7%. No ether could be isolated when a 10-g. sample of the adduct was hydrolyzed. These adducts then were shown to be contaminated with lithium benzoate by total lithium analysis as lithium sulfate and by acid hydrolysis to benzoic acid and benzophenone in quantities indicated by the analysis. Only when an excess of phenyllithium was used could an adduct be obtained for which the lithium content determined by titration agreed with that determined as the sulfate.

The adduct is insoluble in ether or pentane. It is stable over long periods of time in nitrogen atmosphere but is rapidly hydrolyzed to benzophenone when exposed to air. When the dry salt was heated in a sealed tube for 12 hours at 100°, a negligible quantity of benzophenone was isolated by ether extraction.

Reactions of Halides with the Dilithium Salt.—A mixture of 10.3 g., 0.049 mole, of the adduct, 25.8 g., 0.2 mole, of distilled benzyl chloride and 0.8 g. of triethylamine catalyst were heated under nitrogen with stirring. Because no noticeable change occurred at 100°, the temperature was raised to 145° for 45 minutes and finally to 180°. At this point the mixture was a viscous semi-solid. No water was added. The mass was cooled, extracted with dry pentane and filtered. Fractionation of the pentane through an 18-plate column gave 15.9 g. of recovered benzyl chloride, b.p. 63° at 15 mm., $n^{20}D$ 1.5390, and 11.1 g. of liquid product, b.p. 79–174° at 15 mm. Refractionation of the latter liquid at 1–2 mm. gave eight fractions, $n^{20}D$ 1.5800–1.5890. This material consisted of four compounds which were separated by chromatography on alumina columns. Obtained were: benzophenone, m.p. 48–49°; 2,4-dinitrophenylhydrazone, m.p. 239°[8]; benzaldehyde, 2,4-dinitrophenylhydrazone m.p. and mixed m.p. 235–236.5°[9]; stilbene, m.p. 123–124°, dibromo derivative m.p. 235–236.5°[10]; dibenzyl ether, infrared spectral peaks at 3.33, 3.55, 6.25, 6.30, 6.68, 7.37, 8.08, 8.30, 9.02, 9.11, 9.32, 9.73, 12.70, 13.17, 13.41, 13.65 and 14.37; autoxidation to benzaldehyde after seven days.[4] The mixture contained about 35% of benzophenone as shown by a quantitative precipitation of its 2,4-dinitrophenylhydrazone.[11] No dibenzyl ketal of benzophenone, b.p. 305° at 40 mm., m.p. 103–105°, could be detected.

Similar reactions were carried out in which the adduct was treated with ethyl bromide, methyl iodide, dimethyl sulfate, ethylene bromide and oxalyl chloride. In each case benzophenone was the only compound isolated in more than trace amounts. Ketals could not be detected even when questionable fractions were subjected to hydrolysis and a search made for the corresponding alcohols. The reaction with oxalyl chloride occurred at room temperature with evolution of carbon dioxide and carbon monoxide.

***n*-Butyl Trifluoromethyl Ketone.**—To 64.2 g., 0.54 mole, of lithium trifluoroacetate suspended in 100 ml. of dry ether at 5° was added a solution of 0.54 mole of *n*-butyllithium[12] in 320 ml. of ether. The mixture became very viscous but returned to a more fluid state when heated. After 11 hours of stirring and refluxing, the complex was decomposed by the addition of 150 ml. of cold concentrated hydrochloric acid. This addition was made rapidly with stirring over a period of five minutes. Fractionation through a 12-plate column gave 50.3 g., 61%, of *n*-butyl trifluoromethyl ketone, b.p. 29–32° at 65 mm., and 7.7 g. of material, b.p. 69.5° at 8.5 mm., from which the amide of *n*-valeric acid, m.p. 114–116°,[13] the *p*-phenylphenacyl ester of this acid, m.p. 68°,[14] and the semicarbazone of di-*n*-butyl ketone, m.p. 89–90°,[15] were prepared. Refractionation of a portion of the main product gave *n*-butyl trifluoromethyl ketone, b.p. 90° at 740 mm., $n^{20}D$ 1.3410.

Anal. Calcd. for $C_6H_9OF_3$: C, 46.75; H, 5.84. Found: C, 47.15; H, 5.93.

The ketone was cleaved readily by warming with 10% sodium hydroxide solution. A gas was liberated and valeric acid was identified in the residual solution as the amide, m.p. 114–115°.[13]

COLLEGE OF CHEMISTRY AND PHYSICS
THE PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, PENNSYLVANIA

(8) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 264.

(9) Reference 8, p. 229.

(10) W. G. Young, D. Pressman and C. D. Coryell, THIS JOURNAL, **61**, 1644 (1939).

(11) H. A. Iddles, *et al.*, *Ind. Eng. Chem., Anal. Ed.*, **11**, 102 (1939).

(12) H. Gilman, *et al.*, THIS JOURNAL, **71**, 1499 (1949).

(13) H. Weidel and G. L. Ciamician, *Ber.*, **13**, 69 (1880); C. Oddo and E. Calderaro, *Gazz. chim. ital.*, **53**, 71 (1923).

(14) N. L. Drake and J. Bronitsky, THIS JOURNAL, **52**, 3719 (1930).

(15) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, 629 (1912).

Use of the Carboxylic Cation Exchange Resin IRC-50 in the Purification of Thyrotropic Hormone (TSH)

BY JOHN F. CRIGLER, JR.,[1] AND D. F. WAUGH

RECEIVED JUNE 13, 1955

Use of the carboxylic cation exchange resin IRC-50 for the purification of thyrotropic hormone (TSH) was first reported by Heideman.[2] More recently other workers reported that this resin irreversibly adsorbs TSH and have developed a multistep method for obtaining a TSH preparation comparable in specific activity to that described below.[3]

Attempts to repeat the experiments described by Heideman were unsuccessful in that TSH activity was not adsorbed. It was found that the adsorbent should first be equilibrated at paired values of *p*H and ionic strength in order to establish conditions under which TSH activity may be selectively adsorbed. The range investigated comprises *p*H values from 7.0 to 8.0 and corresponding ionic strengths below 0.05 (0.02 to 0.0025 *M*). The protein is dissolved in the same buffer used to equilibrate the resin.

The following procedure is one that has been found satisfactory in using IRC-50 for purifying TSH from beef anterior pituitaries. The resin is prepared in a manner described for XE-64 by Hirs, *et al.*,[4] except that the final equilibration is with 0.01 *M* sodium phosphate buffer at *p*H 7.6 (ionic strength approx. 0.027). A water extract of acetone powder of beef anterior pituitaries[5] containing approximately 0.05 U.S.P. unit per mg. protein (dry weight) was used at a level of 1 U.S.P. unit per 20 ml. of packed resin in a column 30 × 0.9 cm. The column was operated at a rate of approximately 0.5 ml./minute when using a solution containing 1 mg./ml. of protein (dry weight). Following adsorption of TSH activity, the column was washed free of unadsorbed protein with the equilibrating buffer prior to elution with 1 *M* sodium chloride (other cations can be used).

The product obtained represented approximately 5% of the original protein (calculated from ultraviolet absorption) and was found to contain 1.0 to 2.0 U.S.P. units of TSH activity per mg. The assay used employed the uptake of radioactive iodine

(1) Aided by a Fellowship from the National Foundation for Infantile Paralysis.

(2) L. M. Heideman, *Endocrinology*, **53**, 640 (1953).

(3) I. G. Fels, M. E. Simpson and H. M. Evans, *J. Biol. Chem.*, **213**, 311 (1955).

(4) C. H. W. Hirs, S. Moore and W. H. Stein, *ibid.*, **200**, 493 (1953).

(5) Acetone powder of anterior pituitaries was kindly supplied us by Armour and Company through the courtesy of Dr. Sanford Steelman

(I^{131}) in day-old white leghorn chicks[6] fed a low-iodine starter mash,[7] a modification of that described by Shellabarger.[8] The minimally effective dose of the U.S.P. standard by this method is 10 milliunits. Assays were carried out using 2 dose levels of unknowns with 2 or 3 dose levels of the U.S.P. standard, 5 to 8 chicks per group.

Once purified, TSH can be readsorbed providing the excess cations are removed by dialysis, and by this means the specific activity of the final preparation can be further enhanced. The system also has been found to operate in batch preparation and is capable of removing TSH activity from other purified anterior pituitary hormone preparations. The finer mesh carboxylic cation exchange resin XE-64 also has been used but found to possess no apparent advantages over IRC-50 with respect to capacity for TSH activity.

Other investigators[9] working independently have recently reported to us that they have obtained similar reversible adsorption of TSH activity with IRC-50.

(6) White leghorn chicks were obtained from the Hall Bros. Hatchery Inc., of Wallingford, Conn.

(7) Low-iodine starter mash was furnished by the Wirthmore Feeds through the courtesy of Dr. W. A. Glista.

(8) C. J. Shellabarger, *J. Applied Physiol.*, **6**, 721 (1954).

(9) R. W. Bates and P. G. Condliffe, personal communication.

DEPARTMENT OF BIOLOGY
MASSACHUSETTS INSTITUTE OF TECH.
CAMBRIDGE 39, MASS.

The Mode of Hydrolysis of Tetraalkyl Titanates

BY AMEDEO F. D'ADAMO, JR., AND ROY H. KIENLE

RECEIVED APRIL 7, 1955

It was of interest to determine the mode of hydrolysis of the tetraalkyl titanates. The hydrolysis may occur either by cleavage of the C–O– bond by SN1 or SN2 attack or by cleavage of the Ti–O– bond by attack of water along the coördinate axis of titanium.

That SN1 attack does not occur has been shown by Cullinane and co-workers[1] who obtained isobutyl alcohol on hydrolysis of tetraisobutyl titanate. If SN1 attack has occurred, isomerization of the primary carbonium ion would have led to the isolation of *sec*-butyl alcohol.

Due to the nature of the neopentyl system[2] it was felt that investigation of hydrolysis of tetraneopentyl titanate would distinguish between cleavage of the C–O– bond and cleavage of the Ti–O– bond by attack along the coördinate axis of titanium. If the attack of water is along the coördinate axis of titanium, with subsequent breaking of the Ti–O– bond, then the alcohol produced will be neopentyl alcohol. However, if the C–O– bond is broken, then a rearrangement of the carbon skeleton will occur with the formation of isoamyl alcohol.

Accordingly, tetraneopentyl titanate was triturated with water and the alcohol produced separated from the resulting titanium dioxide. The solid alcohol gave an infrared curve identical with that of an authentic sample of neopentyl alcohol. Thus the attack of hydrolytic reagents on titanium alkylates must proceed along the coördinate axis of titanium with subsequent cleavage of the titanium–oxygen bond.

(1) N. Cullinane, *et al.*, *J. Soc. Chem. Ind.*, **69**, S 38–40 (1950).

(2) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 317–318.

Experimental

Materials.—The trimethylacetic acid was obtained from Eastman Kodak Co. and the tetraisopropyl titanate from E. I. du Pont Co.

Neopentyl Alcohol.—Trimethylacetic acid was reduced to neopentyl alcohol with $LiAlH_4$,[3] m.p. 51–53°, in 85% yield.

Tetraneopentyl Titanate.—Neopentyl alcohol (88.15 g., 1.0 mole) and titanium tetraisopropylate (71.0 g., 0.25 mole) were mixed together and allowed to stand overnight.

The mixture was fractionally distilled and a fraction boiling at 130–132° at 1 mm. was collected. This material solidified immediately in the collecting flask. It weighed 62 g. and melted at 58–60°.

Anal. Calcd. for $C_{20}H_{44}O_4Ti$: Ti, 12.1. Found: Ti, 12.7.

Hydrolysis of Tetraneopentyl Titanate.—Tetraneopentyl titanate (7.93 g., 0.02 mole) was triturated with 25 ml. of water for one hour. The mixture was extracted with ether and the ether layer dried over anhydrous sodium sulfate.

After filtering from the sodium sulfate the ether solution was fractionally distilled. On removal of the ether and cooling of the residue, a solid weighing 5.2 g. and melting at 51–53° precipitated out.

Comparison of the infrared curve of this material with the infrared curve of an authentic sample of neopentyl alcohol showed that the two are identical.

Acknowledgment.—The authors are indebted to Mrs. Celia M. Jorgensen for the infrared spectra and Drs. Jason Salsbury and John T. Shaw for their interest in this problem.

(3) R. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 2548 (1947).

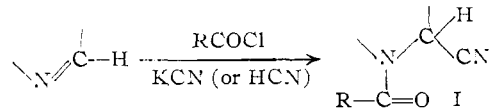
AMERICAN CYANAMID CO., RESEARCH DIVISION
BOUND BROOK, NEW JERSEY

Nature of the Organic Base in Reissert Compounds[1]

BY I. W. ELLIOTT, JR.

RECEIVED MARCH 10, 1955

Reissert compounds (I) result from the addition of an acyl and a cyano group to the azomethine linkage of certain N-heterocyclics.[2] However, Gassman and Rupe[3] found that among a variety of



I

quinoline derivatives there was no easily discernible relation between the electronic nature of the substituent and the ability to form a Reissert compound. The series of quinoline bases studied by Rupe is more noteworthy for the examples of failure than success.[4] In addition to quinoline itself, 6-methoxyquinoline[3] and 7-methoxyquinoline[5] have been

(1) Supported in part by a Cottrell grant from the Research Corporation.

(2) (a) A. Reissert, *Ber.*, **38**, 1603; 3415 (1905); (b) for a complete discussion of "Reissert Compounds" see W. E. McEwen and R. L. Cobb, *Chem. Rev.*, in press.

(3) A. Gassman and H. Rupe, *Helv. Chim. Acta*, **22**, 1241 (1939).

(4) Quinolines with the following substituents did not give the Reissert compound by the method of Rupe: 2-methyl-, 5-nitro-, 5-amino-, 6-dimethylamino-, 7-nitro-, 8-hydroxy-, 8-methoxy-, 8-benzoyloxy- and 8-acetoxy-.

(5) E. Späth and O. Brunner, *Ber.*, **57**, 1234 (1924).