spectra were measured at a concentration of 5 moles/l. in carbon tetrachloride containing a small amount of tetramethylsilane as an internal standard. The spectra were obtained either on a Varian Model DP60 spectrophotometer at 56.4 Mc./sec and calibrated by the method of audio side-band modulation, as previously described,[4] or were measured directly on a Varian A-60 spectrophotometer. The chemical shifts measured either way are believed to be accurate to better than 0.01 p.p.m. The aromatic coupling patterns, where reported, were evaluated by standard treatment. The assignments were made on the basis of the known values of *ortho*, *meta*, and *para* proton coupling constants in aromatic molecules.[52]

Molecular Orbital Calculations. These were made on a Burroughs Model 205 computer using programs especially written for this machine.

Acknowledgments. Special thanks are due to R. L. Feinland for assistance with the preparative v.p.c. separations, to W. H. Jura for the oxidation potential measurements, and to J. E. Lancaster and M. T. Neglia for the n.m.r. measurements. We also wish to express our gratitude to S. M. Kern for writing the computational programs and to D. W. Behnken for valuable mathematical assistance.

(52) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959.

The Exchange Labeling of Keto Steroids with Tritium by Adsorption Chromatography on Basic Alumina[1]

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Contribution from the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois. *Received January 29, 1965*

Keto steroids may be labeled with tritium by chromatography on basic alumina treated with tritiated water. The tritium is not removed by recrystallization in hydroxylic solvents but 95% can be back-exchanged under strongly basic conditions. Reduction of the keto steroids with lithium aluminum hydride occurs without loss of label and provides the corresponding alcohols in which the tritium is no longer exchangeable. The method provides pure, highly labeled products with remarkable economy of tracer.

A number of investigators have reported the use of gas chromatographic systems to carry out isotopic exchange reactions of the type

$$AX + BX^* \longrightarrow AX^* + BX$$

where BX* is a labeled support or stationary phase in the column and AX is the sample containing the exchangeable atom X, which may be halogen or hydrogen.[2-7] Elias[6] has pointed out that the use of a chromatographic system to carry out such exchanges has the inherent advantages of speed, economy, efficiency, and a high purity of the labeled compound which is obtained. To date, however, the catalog of such exchange reactions which have been carried out with organic compounds includes only simple alcohols, alkyl halides, and other compounds which are volatile and stable at the temperature of the gas chromatographic column. Furthermore, the introduction of radioactivity has been limited to those easily exchangeable atoms which, unfortunately, back-exchange readily in unlabeled media and thus have limited utility in tracer or metabolic studies.

This investigation was undertaken with two objectives in mind, to establish the applicability of liquid–solid chromatographic systems to such exchange reactions and to investigate means for the replacement of less labile hydrogens in a molecule. Because of obvious applications to the study of intermediates in sterol metabolism, the replacement of enolic hydrogens in keto steroids during passage through a basic alumina column was selected for these prototypical experiments. The results indicate that it is possible to prepare labeled steroids with tritium contents of 5–10 mc./mmole, whose radioactivity is stable under all conditions short of strong alkali, by this simple procedure.

Experimental

Melting points were observed on a Fisher-Johns heating block and are uncorrected. Optical rotations were measured in chloroform using a Schmidt and Haensch Lippich manual polarimeter. Ultraviolet spectra were run on a Cary Model 11 MS recording ultraviolet spectrophotometer. All compounds reported were purified until they gave a single spot on thin layer chromatography with silica gel HF_{254} (Brinkmann Instruments, Inc.). Chromatoplates were developed in hexane containing various amounts of ethyl acetate and, after drying, were sprayed with ceric sulfate solution (2% in 2 *N* sulfuric acid) and heated in an oven to produce charring.

Column Preparation and Operation. Basic alumina (33 g., Merck, suitable for chromatography) was mixed with 1 ml. of HTO containing 1 c./ml. The deactivated adsorbent was thoroughly stirred and allowed to equili-

(1) Work supported by U. S. Atomic Energy Commission.
(2) F. Schmidt-Bleek, G. Stöcklin, and W. Herr, *Angew. Chem.*, 72, 778 (1960).
(3) G. Stöcklin, F. Schmidt-Bleek, and W. Herr, *ibid.*, 73, 220 (1961).
(4) J. Tadmor, *J. Inorg. Nucl. Chem.*, 23, 158 (1961).
(5) H. Elias, K. H. Lieser, and F. Sorg, *Radiochim. Acta*, 2, 30 (1963).
(6) H. Elias, Proceedings of the Conference on Methods of Preparing and Storing Marked Molecules, Euratom, Brussels, Nov. 13–16, 1963, p. 531.
(7) J. Tadmor, *Anal. Chem.*, 36, 1565 (1964).

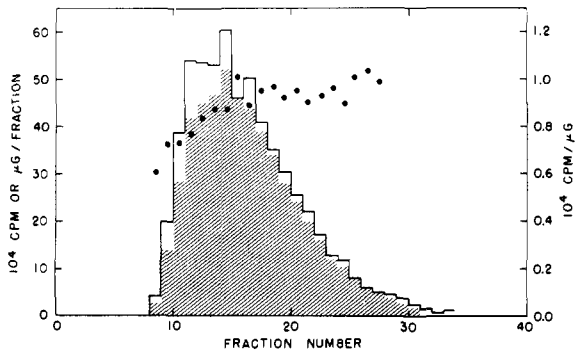

Figure 1. The incorporation of tritium into cholest-7-en-3-one by chromatography on tritiated basic alumina; sample size, 10.0 mg., aliquot 1/20; line, mass of keto steroid; crosshatch region, tritium; dots, specific activity.

brate for 24 hr. before use. The chromatographic column was 6 × 100 mm. and consisted of a Luer tip on 6-mm. i.d. tubing fused to a 24/40 ⅎ female joint which provided a small reservoir above the column bed as well as connection to the larger solvent reservoir. The column was shut off during filling with a sealed needle shank, solvent (50% benzene–pentane, v./v.) was introduced to fill the column, and the adsorbent was sifted in until the proper height of column bed had been obtained. Fresh solvent was passed through the column until a volume of 60 ml. (20 column volumes) had been collected. Chromatographic samples of 0.5–10.0 mg. were applied in a small volume of solvent and elution was carried out at a flow rate of 1 ml./min., collecting 2-ml. fractions. Aliquots were evaporated to dryness[8] in scintillation vials and 10 ml. of scintillation fluid (toluene containing 4% 2,5-diphenyloxazole and 0.4% 1,4-bis-2(5-phenyloxazolyl)benzene) was added. Counting (at 27% efficiency) was done in a Packard EX-2 scintillation spectrometer. Colorimetric analysis of the cholest-7-ene-3-one was carried out using the Liebermann–Burchardt reaction and reading the optical density at 620 mμ after a 5-min. color development at 27°.

Materials. *Cholest-7-en-3β-ol* was prepared by hydrogenation of 7-dehydrocholesterol (Nutritional Biochemicals Corp.) over Raney nickel (W4) in dioxane[9] and crystallized from methanol–acetone as needles, m.p. 124–125°, $[\alpha]^{28}D$ 0° (*c* 1.3) (lit.[9] m.p. 125–126°, $[\alpha]D$ +4°). Acetylation with acetic anhydride and pyridine at room temperature gave *cholest-7-en-3β-ol acetate*, crystallizing as needles from chloroform–methanol, m.p. 119–121°, $[\alpha]^{26}D$ −5° (*c* 1.17) (lit.[9] m.p. 118–119°, $[\alpha]D$ +2.4°). *Cholest-7-en-3-one* was obtained by oxidation of *cholest-7-en-3β-ol* with chromium trioxide in pyridine[10,11] and crystallized from methanol–chloroform as plates, m.p. 147–149°, $[\alpha]^{28}D$ +22° (*c* 0.85) (lit.[11] m.p. 146–147°, $[\alpha]D$ +25°).

4α-Methylcholest-7-en-3-one was obtained by direct methylation of *cholest-7-en-3-one* with methyl iodide and potassium *t*-butoxide in *t*-butyl alcohol.[12] Chromatography on alumina followed by crystallization from methanol–chloroform gave the ketone as needles, m.p. 139–140°, $[\alpha]^{26}D$ +13° (*c* 0.72) (lit.[11] m.p. 139–140°, $[\alpha]D$ +12°).

4,4-Dimethylcholest-7-en-3-one was prepared by the following sequence. *7-Dehydrocholesterol* was oxidized by the Oppenauer procedure to *cholesta-4,7-dien-3-one*,[13] m.p. 89–91°, $[\alpha]^{26}D$ +32° (*c* 0.47), λ_{max} 238 mμ (ε 14,500) (lit.[13] m.p. 86–88°, $[\alpha]D$ +33°, λ_{max} 238 mμ (ε 15,500)). Methylation of the ketone with methyl iodide and potassium *t*-butoxide in *t*-butyl alcohol[14] afforded *4,4-dimethylcholesta-5,7-dien-3-one*, m.p. 160–162°, $[\alpha]^{26}D$ −20° (*c* 0.8) (lit.[14] m.p. 162–163°, $[\alpha]D$ −19°), which was reduced with lithium aluminum hydride to *4,4-dimethylcholesta-5,7-dien-3β-ol*, m.p. 141–143°, $[\alpha]^{25}D$ −163° (*c* 0.40, 2-dm. tube), λ_{max} 273 mμ (ε 10,500) and 282 mμ (ε 10,500) (lit.[14] m.p. 139–141°, $[\alpha]D$ −159°, λ_{max} 282.5 mμ (ε 10,300) and 273 mμ (ε 10,300). Hydrogenation over Raney nickel in dioxane[14] then gave *4,4-dimethylcholest-7-en-3β-ol*, m.p. 145–146°, $[\alpha]^{28}D$ +4° (*c* 1.57), the ultraviolet spectrum of which showed less than 1% diene (ϵ_{283} 65) (lit.[14] m.p. 145–147°, $[\alpha]D$ +5°). Alternatively *4,4-dimethylcholesta-5,7-dien-3-one* could be hydrogenated directly over Raney nickel to *4,4-dimethylcholest-7-en-3β-ol*. Oxidation of *4,4-dimethylcholest-7-en-3β-ol* with 8 *N* chromic acid in sulfuric acid (Jones reagent) then gave the ketone[15] as needles from chloroform–methanol, m.p. 129–130°, $[\alpha]^{26}D$ −29° (*c* 1.99). *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72; mol. wt., 412. Found: C, 84.55; H, 11.70; mol. wt. (mass spectrum), 412.

Cholestane-3,6-dione. Cholest-4-ene-3,6-dione (4.1 g.), prepared according to the method of Fieser[16] by dichromate oxidation of cholesterol, was dissolved in acetic acid (200 ml.), and zinc dust (4.0 g.) was added to the stirred, refluxing solution over a period of 20 min. After a further 25 min. at reflux, the solution was cooled, filtered, and diluted with water. Extraction with ether in the usual way furnished a white solid which was recrystallized from methanol–chloroform as long needles, m.p. 170–173°, $[\alpha]^{26}D$ +2° (*c* 2.15) (lit.[17] m.p. 169–171°, $[\alpha]D$ +5°).

Results

When a sample of cholest-7-en-3-one is applied to a chromatographic column of the type described, the mass and radioactivity eluted from the column by the solvent emerge in the pattern shown in Figure 1. The two peaks coincide, indicating that radioactivity is present in the previously unlabeled ketone. The specific activity is approximately constant but shows a small rise across the chromatogram which is attributable to

(8) Traces of moisture remaining even in freshly distilled solvents result in the elution of tritium as water in the chromatographic fractions. Because of the high specific activity of the HTO, this contributes perceptibly to the background count unless it is removed by evaporation together with the solvent. Alternatively, allowing the samples to stand in the open air permits a fairly rapid equilibration with water vapor in the air which also reduces this effect.

(9) L. F. Fieser and J. E. Herz, *J. Am. Chem. Soc.*, **75**, 121 (1953).

(10) G. I. Poos, G. E. Arth, R. E. Bayler, and L. H. Sarett, *ibid.*, **75**, 422 (1953).

(11) Y. Mazur and F. Sondheimer, *ibid.*, **80**, 6296 (1958).

(12) W. W. Wells and D. H. Neiderheiser, *ibid.*, **79**, 6569 (1957).

(13) R. Antonucci, S. Bernstein, D. Giancola, and K. Sax, *J. Org. Chem.*, **16**, 1453 (1951).

(14) F. Gautschi and K. Bloch, *J. Biol. Chem.*, **233**, 1343 (1958).

(15) Analysis by Dr. A. Bernhardt, Mulheim, Germany. We are indebted to Dr. Carl Djerassi of Stanford University for the mass spectrum. This compound has been reported by P. Witz, H. Hermann, J. M. Lehn, and G. Ourisson, *Bull. soc. chim. France*, 1101 (1963). No physical constants were given.

(16) L. F. Fieser, "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963–1964, p. 189.

(17) L. Caglioti, G. Cainelli, and G. Maina, *Tetrahedron*, **19**, 1057 (1963).

the progressively longer residence time on the column of successive fractions. This results in an increase in the degree of equilibration and consequently of exchange.

The material from one such chromatogram was collected and the solvent was evaporated. After recrystallization, the product weighed 7.15 mg. and had a specific activity of 5036 c.p.m./μg., m.p. 143–145° (starting material 145–147°). It was diluted with 18.0 mg. of unlabeled ketone (new specific activity 1430 c.p.m./μg.) and reduced in ether solution with lithium aluminum hydride. The recovered product was precipitated with digitonin and, upon cleavage of the digitonide, 21.7 mg. of cholest-7-en-3β-ol was obtained with specific activity of 1410 c.p.m./μg; 17.4 mg. of the crude sterol was recrystallized from methanol yielding in the first crop 8.8 mg., m.p. 123–125° (lit.[9] 125–126°), whose specific activity was 1440 c.p.m./μg. An aliquot was acetylated with acetic anhydride in pyridine and the recovered sterol acetate was mixed with a reference sample of cholest-7-en-3β-ol acetate-1-C^{14}. The mixture of H^3 and C^{14} samples was chromatographed on a Davison Code 12 silica gel column according to the method of Klein and Szczepanik.[18] The chromatographic identity of the C^{14}-labeled reference material and the material obtained by isotopic tritium exchange (and the latter's homogeneity) is demonstrated in Figure 2. Isotopic fractionation of the H^3-labeled form from the C^{14} form was computed by the three alternative methods described by Klein, Simborg, and Szczepanik.[19] These data, shown in Table I, indicate a consistent and significant fractionation effect comparable to that previously obtained[18] for cholesterol acetate-1-C^{14} and cholesterol acetate-2-H^3.

Table I. Chromatographic Characteristics of H^3-Labeled Cholest-7-en-3β-ol Acetate and Cholest-7-en-3β-ol Acetate-1-C^{14}

	C^{14} reference	H^3 exchange labeled
Retention volume[a] (fraction number)	140.57 ± 0.05[b]	141.03 ± 0.06
Band width[a] (σ)	8.37 ± 0.05	7.98 ± 0.06
Displacement of H^3 from C^{14} as per cent of C^{14} mobility		ΔM, %
By probit analysis		0.516 ± 0.055
From H^3/C^{14} ratio, neglecting differences in dispersion		0.720 ± 0.102
From H^3/C^{14} ratio, correcting for differences in dispersion		0.724 ± 0.068

[a] Computed from probit analysis of cumulative per cent eluted.[19] [b] Standard error of the mean.

The location of the label was inferred from the series of experiments shown in Table II. When the labeled ketone was dissolved in refluxing methanol and the solvent evaporated *in vacuo*, no perceptible loss of radioactivity took place, indicating that easily exchangeable tritium was absent. On the other hand, when an aliquot was refluxed with methanolic KOH and recrystallized, more than 95% of the label was removed. The persistence of tritium in the sample after this treatment suggested that a small amount of label might have been introduced elsewhere in the molecule, possibly in allylic positions about the Δ^7 bond. Accordingly, a sample of cholest-7-en-3β-ol acetate was chromatographed on the same column and recrystallized from methanol. This compound possesses no enolic hydrogens and the residual labeling of 20.5 c.p.m./μg. indicated that a certain contribution of non-enolic substitution was indeed present. This was further confirmed by chromatography of the saturated ester, cholestan-3β-ol acetate. After recovery of this product, a labeling of 0.89 c.p.m./μg. was obtained. This low level may be attributable to Wilzbach labeling or to the presence of essentially infinitesimal traces of impurities.

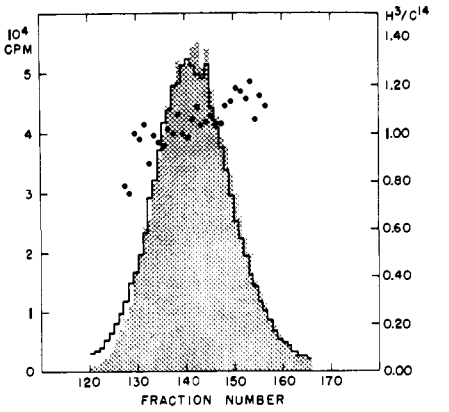

Figure 2. The chromatographic purity and identity of exchange-labeled cholest-7-en-3β-ol acetate with pure C^{14}-reference acetate; column, Davison Code 12 silica gel (100–200 mesh) equilibrated at 7.05% humidity and elution with 16% benzene in pentane; flow rate, 24 ml./hr. (fraction size, 6.0 ml.): solid line, C^{14}; crosshatched area, H^3; dots, H^3/C^{14}.

Although no extensive tests of column efficiency and durability are reported here, it has been our experience that a given column, after several preliminary runs, will give a consistent labeling activity for a number of runs before it becomes inactivated or depleted by the residual traces of moisture in the eluting solvents. Such consistent incorporation is demonstrated by the replicate and successive products shown in Table III. The variation (such as exists) between runs may well be attributable to differences in loading and flow rates during the operation of the column, but is not unreasonable in extent in any case.

If, for a given column, successive runs do indeed provide comparable labeling, one may study the effect of structure of the ketone on the extent of labeling achieved during the chromatography. Such a study is shown for several alumina columns in Table IV, which indicates that the specific activity of the ketone is related, though not with direct proportionality, to the number of enolic hydrogens in the molecule. When substituents are present on C-4, the extent of labeling is decreased, whereas the presence of two keto groups in the same molecule markedly enhances the degree of tritium incorporation.

It was of interest to determine if the tritium content of exchange-labeled cholest-7-en-3-one could be reduced by chromatography on unlabeled alumina columns. Figure 3 shows that depletion of the label

(18) P. D. Klein and P. A. Szczepanik, *J. Lipid Res.*, 3, 460 (1962).
(19) P. D. Klein, D. W. Simborg, and P. A. Szczepanik, *Pure Appl. Chem.*, 8, 357 (1964).

Table II. Tritium Content of Steroids after Various Treatments

Compd.	Wt., mg.	Treatment	Specific activity, c.p.m./μg.	Relative activity, %
Cholest-7-en-3-one	10.0	Chromatographed on tritiated alumina	2972	(100)
	1.0	Recrystallized from hot methanol	3166	106.5
	0.83	Refluxed with 10% KOH in methanol, recrystallized from methanol	123.8	4.1
Cholest-7-en-3β-ol acetate	9.0	Chromatographed on tritiated alumina, recrystallized from methanol	20.5	0.69
Cholestan-3β-ol acetate	9.35	Chromatographed on tritiated alumina, recrystallized from methanol	0.89	0.029

occurs during passage through such a column and, in contradistinction to the forward process, the specific activity progressively *decreases*, again substantiating the correlation between residence time and the degree of equilibration. The tritium content of the product was reduced to 51% of the initial activity by passage

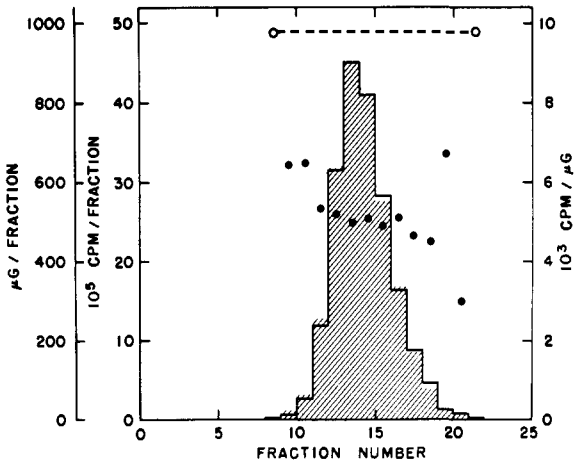

Figure 3. The removal of tritium from labeled cholest-7-en-3-one by chromatography on unlabeled basic alumina (symbols as in Figure 1): open circles, specific activity of original material.

through a column which was of the same dimensions, size, and activity as the labeling column. In all likelihood, the incomplete back-exchange is a consequence of the greater stability of the C–T bond as opposed to the C–H bond involved in the enolization process.

Discussion

The highest specific activities which have been achieved to date with this batch of adsorbent have been 9500 c.p.m./μg. of cholest-7-en-3-one and 13,500 c.p.m./μg. of cholestane-3,6-dione. These specific activities correspond to 6.6 and 9.7 mc./mmole, respectively, and are less than the 18 mc./mmole of the water used to prepare the adsorbent. Several considerations mitigate against a direct computation at this time of the exchange efficiency from these values. These are (a) the unknown extent to which the specific activity of the HTO was diluted by exchangeable hydrogen on the alumina surface, and (b) an inability to specify the number of enolic hydrogens actually accessible for exchange when the molecule is oriented by adsorption on the surface.

Table III. Incorporation of Tritium in Replicate Chromatograms on the Same Column

Cholest-7-ene-3-one		
Sample wt. applied, mg.	Wt. recovered, mg.	Specific activity, c.p.m./μg.
10.4	10.5	5524
1.00	1.02	6228
2.00	1.80	6770
0.50	0.65	5814
4.00	3.93	5226
	Av.	5712

Table IV. Effect of Keto Steroid Structure on Tritium Incorporation during Chromatography

Compd.	Enolic positions	Wt. applied, mg.	Specific activity, c.p.m./μg.	Relative activity, %
Cholest-7-en-3-one	4	3.93	5526	(100)
4,4-Dimethylcholest-7-en-3-one	2	11.13	1172	22.4
Cholest-7-en-3-one	4	1.0	2400	(100)
4α-Methylcholest-7-en-3-one	3	11.55	542	22.6
4,4-Dimethylcholest-7-en-3-one	2	10.50	507	21.1
Cholest-7-en-3-one	4	1.0	3414	(100)
Cholestane-3,6-dione	7	10.4	13572	397

The use of enolic exchange is a long-standing technique in the preparation of isotopically substituted sterols[20] and has been used by Lindberg, Gautschi, and Bloch[21] to prepare tritium-labeled sterols with specific activities of approximately 1 mc./mmole. The stability of the cholest-7-en-3β-ol acetate during passage through the column described here, however, points up a further advantage of this procedure over the alkali-catalyzed exchange commonly used; namely that ester linkages in the molecule need not be destroyed. Additionally, the alkali-catalyzed exchange is wasteful of tracer since the alkaline solution can seldom be recovered without dilution after removal of the sample.

These experiments demonstrate that exchange labeling by liquid–solid adsorption chromatography is an expeditious, convenient, and efficient method of preparing labeled compounds. The use of basic alumina to promote the enolization of hydrogen atoms adjacent to keto groups enables the insertion of tritium into

(20) M. Anchel and R. Schoenheimer, *J. Biol. Chem.*, **125**, 23 (1938).
(21) M. Lindberg, F. Gautschi, and K. Bloch, *ibid.*, **238**, 1661 (1963).

positions much more resistant to solvent exchange than in those compounds previously labeled by gas chromatographic means. It also provides access to labeling possibilities for those compounds which cannot be volatilized or which would be damaged by the temperatures of the gas chromatographic column. Perhaps the greatest immediate advantage over gas chromatographic labeling techniques is the sample capacity of liquid–solid adsorption columns which extends to gram quantities without special equipment.

Electron Paramagnetic Resonance Studies on Chelation of Alkali Cations by the *o*-Dimesitoylbenzene Radical Anion

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Contribution from the Laboratório de Química O ânica do Instituto Superior Técnico, Lisbon, and the Laboratório de Física e Engenharia Nucleares, Sacavém, Portugal.
Received January 6, 1965

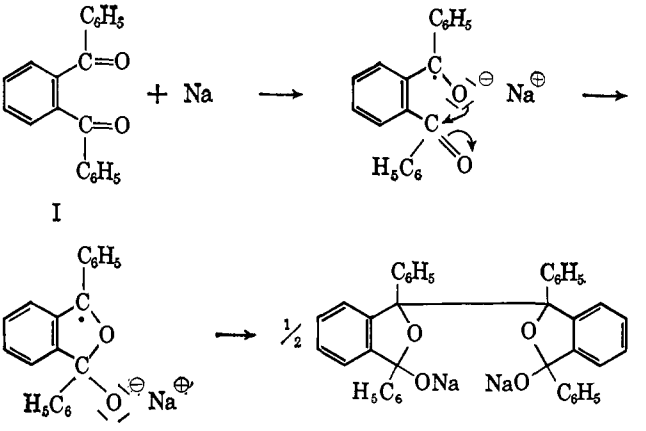
The stable radicals obtained by addition of alkali metals to o-dimesitoylbenzene in 1,2-dimethoxyethane (DME) and tetrahydrofuran (THF) were studied by electron paramagnetic resonance (e.p.r.). A spectrum arising from the interaction of the unpaired electron with one alkali metal nucleus (quadruplet for Li, Na, and K and octuplet for Cs) and from two equivalent protons was observed. In the system o-dimesitoylbenzenepotassium in THF, finer structure from the mesityl groups consistent with considerable noncoplanarity was resolved. The unusually high splittings a_M due to the alkali metal are interpreted in terms of the formation of a chelate with partially covalent bonding. The fraction a_M/a (in which a is the hyperfine splitting constant for the free atom in the fundamental state) is shown to be a linear function of $r^{-1/2}$, r being the ionic radius of the metal. This correlation can be used to predict the alkali metal splitting and shows that the covalent character of the bond increases in the order Cs < K < Na < Li. For this type of complex in which the ligand is a radical the e.p.r. is shown to be a highly satisfactory method to reveal covalency of the bond to the metal.

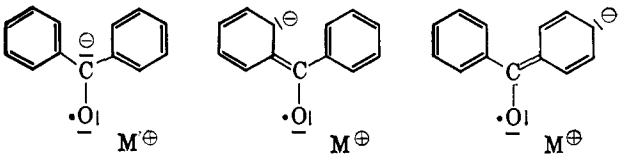
Introduction

In previous studies of the 1:1 addition of sodium to *o*-dibenzoylbenzene (I) in ether (in dry nitrogen atmosphere)[3] it was found that a dimerization occurs. A previous cyclization was postulated.

Although the present investigation does not answer the question as to whether this mechanism is correct, it was undertaken as a first approach to this problem. In the reaction of alkali metals with similar ketones in which the phenyl groups are substituted by bulkier groups, steric hindrance is expected to lessen the equilibrium constant for the dimerization of the radical.

Earlier research in this field has given experimental evidence that the alkali metal chosen is also one of the factors that influence the stability of the ketyls. In terms of resonance theory,[4] in the case, *e.g.*, of benzophenone ketyl, the following structures contribute to the stabilization.





Such structures should have an increasing contribution when the covalent character of the OM bond decreases, which is to be expected in the case of the series Li, Na, K, Rb, and Cs, according to the views of Fajans[5] on the polarizing abilities of ions of different radii. This explains the observation of E. Müller, *et al.*,[6] and Mikhailov[7] that the equilibrium constant for the dimerization of a ketyl decreases in such a series.

Variations in the stability of the ketyl, however, are only an indirect approach to the study of the character of the ligand–metal bond.

(1) Supported in part by the NATO Research Grants Program (182).
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(3) B. J. Herold, *Tetrahedron Letters*, **2**, 75 (1962).
(4) G. W. Wheland, "Advanced Organic Chemistry," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., 1960, p. 796.
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(6) E. Müller and W. Janke, *Z. Elektrochem.*, **45**, 380 (1939).
(7) B. M. Mikhailov and N. G. Chernova, *Dokl. Akad. Nauk SSSR*, **85**, 341 (1952).