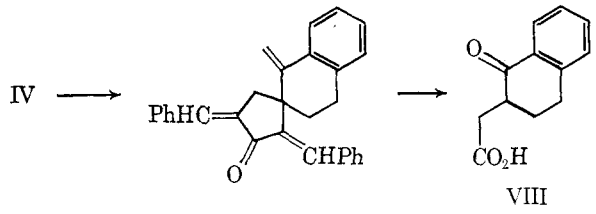
Treatment of IV with benzaldehyde and base gave two isomeric dibenzal derivatives, mp 136.5–138.5°; 5.94, 6.19, and 11.18 μ; $\lambda_{max}^{95\%\ EtOH}$ 239 mμ (ϵ 24,000) and 347 mμ (ϵ 30,100); mol wt 388; and mp 173–175°; 5.93, 6.17, and 11.10 μ; $\lambda_{max}^{95\%\ EtOH}$ 239 mμ (ϵ 20,400) and 347 mμ (ϵ 30,400); mol wt 388). Ozonolysis



VIII

of each dibenzal derivative separately gave VIII, isolated as the 2,4-dinitrophenylhydrazone which was compared with an authentic sample.

The rearrangement of I to II proceeds *via* an excited triplet state.[1b,7] The reaction proceeds at a comparable rate in the presence of dibenzothiophene as sensitizer (greater than 90% of the light absorbed by dibenzothiophene) and in the absence of the sensitizer. Furthermore, the reaction is almost completely quenched by 0.03 *M* piperylene.[8]

Complete resolution of I and II was accomplished *via* the corresponding pyrrolidinium *d*-camphor-10-sulfonates, a new method for resolution of racemic ketones.[9] The rotations of the ketones were I, $[\alpha]^{27}D$ +332 and −332°, and II, $[\alpha]^{27}D$ +42.5 and −42.3°. Table I summarizes the results of irradiation of the

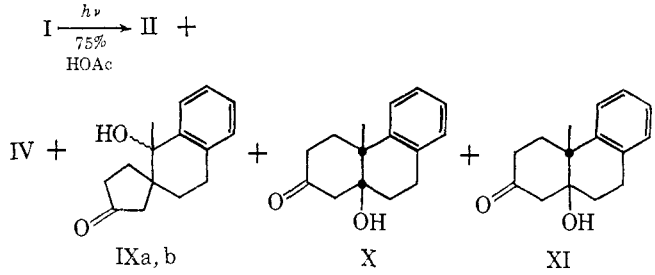
Table I. Summary of Irradiations of Optically Active I[a]

Run	$[\alpha]^{27}D$, deg I	$[\alpha]^{27}D$, deg II	Duration of irradiation, hr[b]	Yield of II, %	Retention of opt. act., %
1	+332	−40.2	5	65	95
2	+332	−40.5	18.5	27	96
3	−332	+40.9	6	80	96
4	−332	+41.0	18.5	37	96

[a] All rotations were measured at *c* 1. [b] All irradiations were carried out in *t*-butyl alcohol at 28–30° using a 550-w Hanovia type A mercury lamp filtered through Pyrex.

enantiomers of I. The rotations of the product were taken on the solid product II after chromatography on silica gel and treatment with charcoal in solvent, but without crystallization. The rotation of II is opposite in sign to that of I so that slight contamination of II with I would lower the apparent retention of optical activity. In all runs using both enantiomers a minimum of 95% retention of optical activity was observed. The photochemical rearrangement thus is stereospecific, and mechanisms which involve complete fission of the 1,10 bond with concomitant formation of an intermediate having a plane of symmetry (*e.g.*, III) can be excluded for the photochemical rearrangement of I to II.

Irradiation (λ >290 mμ) of I in aqueous acetic acid (75%) gives several new products, IXa,b (30%),



X (1%), and XI (3%), in addition to II (<2%) and IV (5%). Control experiments show that IXa,b and IV are produced from II in the dark under the conditions of the experiment. Control experiments suggest that X and XI are derived from I in a photochemical step, but the small amounts of these compounds in the crude product render this conclusion tenuous. The structures of IXa,b (2.88 and 5.75–5.80μ), which we have been unable to separate, rest on dehydration by phosphorus oxychloride in pyridine to IV, which has been degraded to a known compound. Keto alcohols X and XI were identified by comparison with authentic samples.

The relationship of the photochemistry of the cross-conjugated cyclohexadienones[2,4d,10] to the photochemistry of the 2-cyclohexenones is now more clearly in focus. The two series show structurally similar types of rearrangement (alkyl shifts) and similar stereospecificity and nucleophilic solvent incorporation (in the case of the cyclohexenones, at least, this is often the result of a subsequent dark reaction).[4] They differ substantially in quantum efficiency[1b,7] and the rate of reaction of the triplet (*i.e.*, triplets of 2-cyclohexenones can be quenched while those from cyclohexadienones cannot).[1b,7]

Acknowledgment. This investigation was supported by Public Health Service Research Grant AM0720 from the National Institute of Arthritis and Metabolic Diseases.

(7) H. E. Zimmerman, *J. Pure Appl. Chem.*, **9**, 493 (1964). See also ref 1b.

(8) This quenching involves reaction with piperylene in addition to energy transfer.

(9) W. R. Adams, O. L. Chapman, J. B. Sieja, and W. J. Welstead, Jr., *J. Am. Chem. Soc.*, **88**, 162 (1966).

(10) For reviews see (a) P. J. Kropp, chapter in "Organic Photochemistry," Vol. I, O. L. Chapman, Ed., Marcel Dekker, Inc., New York, N. Y., in press; (b) P. de Mayo, *Advan Org. Chem.* **2**, 367 (1960); (c) O. L. Chapman, *Advan. Photochem.*, **1**, 323 (1963); (d) see ref 1f.

(11) Public Health Service Fellow (5-Fl-GM-21, 687-02) National Institute of General Medical Sciences.

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A New Method for the Resolution of Ketones

Sir:

Resolution of racemic ketones and aldehydes, though of frequent interest in mechanism studies and synthesis, remains a difficult problem. Several approaches to this problem have been suggested, prominent among which are optically active carbonyl reagents such as hydrazines,[1] semicarbazides,[2] diols,[3] dithiols,[4] semi-

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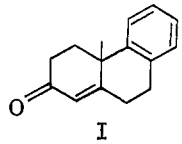
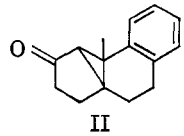
oxamazides,[5] acid hydrazides,[6] carbamates,[7] amine bisulfites,[8] and second-order methods such as reduction to the alcohol (which introduces another optical center) followed by resolution and reoxidation. These methods all suffer from one or more of the following difficulties: (1) tedious preparation of an optically active reagent which may or may not give a crystalline derivative; (2) stringent hydrolysis conditions for regeneration of the carbonyl component which may cause rearrangement or other undesirable reaction; (3) production of a new optical center; (4) failure to produce crystalline derivatives.

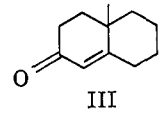
We now wish to describe an approach to the resolution of ketones which eliminates the need to synthesize optically active reagents and which permits regeneration of the carbonyl compound under exceptionally mild conditions (dilute base at room temperature for 5 min). This method has been used to obtain both optically pure enantiomers of I and II. In addition, III and IV have been partially resolved. The method consists of the formation of iminium salts containing optically active anions. Two general procedures were used, procedure A for carbonyl compounds which form enamines readily[9] and procedure B for carbonyl compounds which form enamines only slowly or not at all.[10]



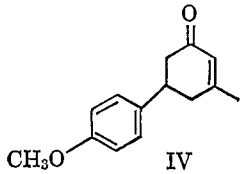
I



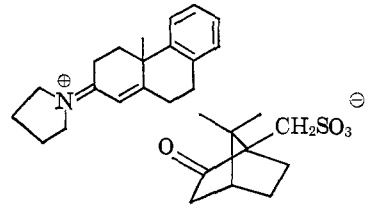
II



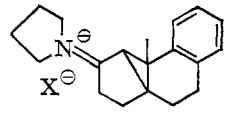
III



IV



V



VI, X = ClO_4

VII, X = d-camphor-10-sulfonate

Procedure A. This procedure is exemplified by the resolution of I. Addition of *d*-camphor-10-sulfonic acid (10.5 g, 0.045 mole) in hot acetone (35 ml) to the pyrrolidine enamine of I[11] (11.7 g, 0.044 mole) in hot acetone (70 ml) gave, after cooling in ice, an iminium salt (V, 19.5 g, 88%). Systematic recrystallization of V from ethyl acetate–methanol gave 4.6 g of salt, mp 163–167°, $[\alpha]^{27}D$ −131° (*c* 1, 95% ethanol), and 4.6 g of salt, mp 167–180°, $[\alpha]^{27}D$ +168° (*c* 1, 95% ethanol). Hydrolysis of levorotatory V (4.2 g) in dilute aqueous potassium hydroxide (100 ml of 0.1 *N*) at room temperature for 5 min gave levorotatory I (1.7 g). Recrystallization from pentane gave levorotatory I, mp 68–69°, $[\alpha]^{27}D$ −332° (*c* 1, 95% ethanol). Hydrolysis of dextrorotatory V gave dextrorotatory I, mp 68–69°, $[\alpha]^{27}D$ +332° (*c* 1, 95% ethanol). The infrared spectra (KBr) of the enantiomers were superimposable. The infrared spectrum (KBr) of the racemate (racemic compound) shows minor differences below 11μ, but very pronounced differences at longer wavelengths. No trace of racemate absorption could be detected in the infrared spectrum (KBr) of either pure enantiomer.

Procedure B. This procedure is exemplified by the resolution of II. To a solution of II in hot absolute ethanol (7.7 g, 0.036 mole in 45 ml) was added pyrrolidine perchlorate (6.4 g, 0.037 mole) and pyrrolidine (2 drops).[10,12] After 3 hr at room temperature the crystals of VI (mp 177–181°, 12.5 g, 93%) were collected and washed with ethanol. A solution of VI (12.5 g, 0.034 mole) and potassium *d*-camphor-10-sulfonate (8.9 g, 0.033 mole) in absolute methanol was stirred for 13 hr. The precipitated potassium perchlorate was filtered off, and the methanol was evaporated. Addition of acetone (10 ml) to the residue precipitated more potassium perchlorate, which was removed. Removal of the acetone, addition of ethyl acetate to the residue, and subsequent scratching produced crystals of VII. Systematic recrystallization of VII from ethyl acetate–methanol gave 0.9 g of salt, mp 128–131°, $[\alpha]^{27}D$ +39.0° (*c* 1, 95% ethanol), and 0.5 g of salt, mp 117–122°, $[\alpha]^{27}D$ 0.00° (*c* 1, 95% ethanol). Both salts analyzed for a monohydrate and both showed strong absorption at 2.88 μ in their infrared spectra. Hydrolysis of dextrorotatory VII (as in procedure A) and recrystallization of the product gave dextrorotatory II, mp 110–111°, $[\alpha]^{27}D$ +42.5° (*c* 1, 95% ethanol). Hydrolysis of levorotatory VII gave levorotatory II, mp 109.5–111.5°, $[\alpha]^{27}D$ −42.3° (*c* 1, 95% ethanol). The infrared spectra (KBr) of the enantiomers were superimposable. The infrared spectrum (KBr) of the racemate (racemic compound) shows relatively small shifts in its absorption bands compared to that of the enantiomers but pronounced differences in the relative intensities of its bands.

10-Methyl-$\Delta^{1,9}$-octalone-2 (III) was partially resolved using method A. The enamine (prepared by the method of Stork[10]; bp 160–170° (15 mm); 17.1 g, 0.084 mole) and *d*-camphor-10-sulfonic acid (19.5 g, 0.084 mole) were dissolved in ethyl acetate and cooled, giving a crystalline iminium salt (18.8 g, 50%), mp 170–172°. Fractional recrystallization (4–5 times) of

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(4) E. J. Corey and R. B. Mitra, *J. Am. Chem. Soc.*, **84**, 2938 (1962).

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(9) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *ibid.*, **85**, 207 (1963).

(10) N. J. Leonard and J. V. Paukstelis, *J. Org. Chem.*, **28**, 3021 (1963).

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(12) Appropriate precautions must always be taken with perchlorates. This is especially true when they are in contact with alcohols.

the salt in ethyl acetate gave partial separation of the two diastereomers. Fractions with mp 176–178°, $[\alpha]^{27}D$ +36.0° (*c* 1, 95% ethanol, 0.4 g), and mp 168–171°, $[\alpha]^{27}D$ +20.0° (*c* 1, 95% ethanol, 0.2 g) were obtained. Hydrolysis of the two iminium salt fractions gave two liquid ketone fractions with $[\alpha]^{27}D$ +26.4° (*c* 1, 95% ethanol, 0.16 g) and $[\alpha]^{27}D$ −3.4° (*c* 1, 95% ethanol, 0.06 g).

Partial resolution of 3-methyl-5-(*p*-methoxyphenyl)-2-cyclohexen-1-one (IV) was achieved using procedure B.

Pyrrolidine perchlorate (1.6 g, 0.009 mole) and IV (2.0 g, 0.009 mole) in absolute ethanol (10 ml.) were cooled, giving the iminium perchlorate salt (1.8 g, 60%), mp 90–91°. Treatment with potassium *d*-camphor-10-sulfonate converted the iminium perchlorate salt to the *d*-camphor-10-sulfonate salt, mp 171–172°. The diastereomeric mixture (16.6 g) was fractionally recrystallized from acetonitrile–ether giving two salt fractions, mp 174–175°, $[\alpha]^{27}D$ +42.3° (*c* 1, 95% ethanol, 4.7 g.), and mp 140–143°, $[\alpha]^{27}D$ −18.0° (*c* 1, 95% ethanol, 0.3 g). Hydrolysis of the two iminium salt fractions gave two crystalline ketone fractions, mp 58–59°, $[\alpha]^{27}D$ +55.6° (*c* 1, 95% ethanol, 1.8 g), and mp 57–59°, $[\alpha]^{27}D$ −26.8° (*c* 1, 95% ethanol, 0.08 g).

The method for resolution presented above is not a panacea. It is, however, the simplest method to try. It does not involve synthesis of optically active reagents, and regeneration of the carbonyl compound is performed under exceptionally mild conditions. The iminium salts are readily available and generally high melting solids.

Satisfactory analyses were obtained for all new compounds.

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(13) DuPont Teaching Fellow, 1964–1965.
(14) Public Health Service Fellow (5-F1-GM-21, 687-02), National Institute of General Medical Sciences.

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Insulin Peptides. XI. The Synthesis of the B Chain of Human Insulin and Its Combination with the Natural A Chain of Bovine Insulin to Generate Insulin Activity[1]

Sir:

In previous communications[2,3] we have reported the synthesis and isolation in the S-sulfonate form of the A and B chains of sheep insulin. Combination experiments between these chains and crossed recombination experiments between the synthetic chains and the natural chains of bovine insulin led to generation of insulin activity. We wish to report now the synthesis and isolation in the S-sulfonate form of the B chain of human insulin. The proposed[4] structure of human insulin is shown in Chart I. The synthetic B chain, upon combination with the natural A chain of bovine insulin, generated insulin activity in an over-all yield ranging from 4 to 8% (based on crystalline bovine insulin). This represents the partial synthesis of a human protein.

N^{α}-Carbobenzoxy-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester [mp 99–101°; $[\alpha]^{27}D$ +1.9° (*c* 10, DMF[5]) (*Anal.* Calcd for $C_{26}H_{35}N_3O_8S$: C, 56.8; H, 6.42; N, 7.7. Found: C, 57.2; H, 6.52; N, 7.9); after hydrogenolysis in the presence of HCl: R_f[6] 0.71], prepared from N^{α}-carbobenzoxy-N^{ϵ}-tosyl-L-lysine p-nitrophenyl ester[7] and threonine methyl ester, was decarbobenzoxylated by catalytic hydrogenation and condensed with N-carbobenzoxy-L-proline *p*-nitrophenyl ester[8] to give N-carbobenzoxy-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (I): mp 125–128°; $[\alpha]^{27}D$ −24.9° (*c* 1.0, DMF) (*Anal.* Calcd for $C_{31}H_{42}N_4O_9S$: C, 57.6; H, 6.55; N, 8.7. Found: C, 57.6; H, 6.67; N, 8.9); after hydrogenolysis in the presence of HCl: R_f[6] 0.68, R_f[9] 4.35 × His. Removal of the carbobenzoxy group from I by hydrogenolysis and coupling of the ensuing product with N-carbobenzoxy-L-threonine[10] by the carbodiimide method[11] yielded N-carbobenzoxy-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (II): mp 84–90°; $[\alpha]^{27}D$ −32.7° (*c* 1, DMF) (*Anal.* Calcd for $C_{35}H_{39}N_5O_{11}S$: C, 56.2; H, 6.60; N, 9.4. Found: C, 56.3; H, 6.56; N, 9.1); for the hydrochloride: R_f[6] 0.72, R_f[9] 5.07 × His. N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (III) [mp indefinite 88–110°; $[\alpha]^{29}D$ −32.1° (*c* 2, DMF) (*Anal.* Calcd for $C_{51}H_{64}N_6O_{13}S$: C, 61.2; H, 6.44; N, 8.4. Found: C, 61.0; H, 6.43; N, 8.4); after hydrogenolysis in the presence of HCl: R_f[6] 0.73, R_f[9] 2.99 × His] was prepared by coupling N-carbobenzoxy-O-benzyl-L-tyrosine *p*-nitrophenyl ester[8] with the product obtained by catalytic hydrogenation of II. Removal of the carbobenzoxy and benzyl groups from III by hydrogenolysis and coupling of the ensuing product with N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester[12] yielded N-carbobenzoxy-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (IV): mp 139–141°; $[\alpha]^{27}D$ −40.9° (*c* 1, DMF) (*Anal.* Calcd for $C_{53}H_{67}N_7O_{14}S$: C, 60.2; H, 6.38; N, 9.3. Found: C, 60.3; H, 6.42; N, 9.2); for the hydrochloride R_f[6] 0.84, R_f[9] 3.96 × His. Catalytic hydrogenation of IV and reaction of the resulting product with N-carbo-

(1) Presented (P. G. K.) at the National Academy of Sciences Autumn Meeting, Seattle, Wash., Oct 11-13 1965, and at the Brookhaven National Laboratory Symposium on "Structure and Function of Polypeptide Hormones: Insulin," Upton, N. Y., Nov 8–11, 1965.
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(4) D. S. H. W. Nicol and L. F. Smith, *Nature*, 187, 483 (1960).
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(6) The R_f refers to the Partridge system: S. M. Partridge, *Biochem. J.*, 42, 238 (1948).
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(9) The R_f refers to the system 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (S. G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953)), and is expressed as a multiple of the distance traveled by a histidine marker.
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