tached to carbons bearing acetoxyl groups. The downfield shift of 1.08 p.p.m. is characteristic of an acetate of a secondary alcohol.[9] These data agree with structure VI, a structure derived from dihydrocembrene II and cembrene I and would not agree with a similar group of structures derived from IV.

Thus, I represents the structure of cembrene. Although no information is available with regard to the stereochemistry of the trisubstituted double bonds, from a study of models the structure written containing one *cis* bond appears to be more favored than an all *trans* structure. This diterpene hydrocarbon, the first naturally-occurring fourteen carbon ring compound, is specially significant from the biogenetic standpoint, since it is the monocyclic diterpene derived from geranylgeraniol in a manner analogous to the formation of the sesquiterpene humulene from farnesol.[10,11]

(9) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 55.

(10) J. B. Hendrickson, *Tetrahedron*, **9**, 82 (1959).

(11) M. D. Sutherland and O. J. Waters, *Aust. J. Chem.*, **14**, 596 (1961).

(12) National Science Foundation Predoctoral Fellow, 1957–1959.

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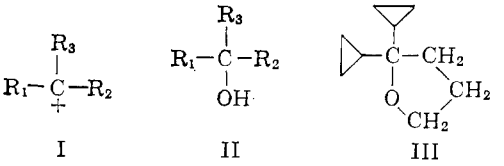
PHYSICAL PROPERTIES OF THE TRICYCLOPROPYLMETHYL CATION[1]

Sir:

Recently the physical properties of a simple aliphatic alkenyl cation were reported.[2] We now report the physical properties of the tricyclopropylmethyl cation, an aliphatic carbonium ion containing no formal double bonds.

Hart and Sandri[3] showed from solvolysis studies that cyclopropyl groups stabilize carbonium ions. The *p*-nitrobenzoates of IIb, IIc, and IId solvolyzed with relative rates of 23,500:246:1, indicating that the effect of cyclopropyl groups was nearly additive.[3]



I II III

a, $R_1 = R_2 = R_3$ = cyclopropyl
b, $R_1 = R_2$ = cyclopropyl, R_3 = isopropyl
c, R_1 = cyclopropyl, $R_2 = R_3$ = isopropyl
d, $R_1 = R_2 = R_3$ = isopropyl
e, $R_1 = R_2$ = cyclopropyl, $R_3 = -CH_2CH_2CH_2OH_2^+$

Addition of IIa to 96% H_2SO_4 produces the tricyclopropylmethyl cation (Ia). This ion has a most remarkable n.m.r. spectrum consisting of a single sharp band (width at half-height about 3.5 cycles) at 6.85 τ (60 mc., benzene capillary reference, 2.73 used to relate benzene to tetramethylsilane). This is in striking contrast to the n.m.r. spectrum of IIa (CCl_4 solution), which consists of a complex multiplet from 8.9–9.4 τ and a complex multiplet from 9.5–9.8 τ with areas in the approximate ratio of 1:3. This reduction of the complex cyclopropyl n.m.r. pattern to a single band is also found in protonated dicyclopropyl ketone, which exhibits a band (with poorly resolved fine structure) at 7.05 τ in 96% H_2SO_4. These observations both support the identification of Ia and show the close relation between carbonium ions and protonated ketones.

IIa was recovered in 63% yield from solutions of Ia in 96% H_2SO_4. The b.p. and both n.m.r. and infrared spectra are identical with those of the original IIa used to form Ia. Addition of a solution of Ia in 96% D_2SO_4 to ice–10% NaOH yielded a CCl_4 extract. The n.m.r. spectrum of this extract was virtually identical with that of the original IIa demonstrating that no rapid H–D exchange had occurred and that IIa was the only water insoluble product derived from drowning Ia. Even slow H–D exchanges are absent because the n.m.r. spectrum of Ia in 96% D_2SO_4 was essentially unchanged after one hour. This eliminates an olefin as a component of the equilibrium and reflects the high energy of cyclopropylidene derivatives.

The initial *i*-factor of IIa in H_2SO_4 was 4.1 in accord with the equation $ROH + 2H_2SO_4 = R^+ + H_3O^+ + 2HSO_4^-$. The infrared spectrum of Ia exhibits bands at 837, 1279, and 1445 ($cm.^{-1}$).[4] In the ultraviolet, λ_{max} for Ia (270 mμ, ϵ 22,000)[5] bears a relation to alkenyl cations (310–335 mμ) that is comparable to the relation between protonated cyclopropyl ketones (dicyclopropyl ketone·H^+ 235 mμ, tricyclanone·H^+ 250 mμ)[6] and protonated unsaturated ketones (4-methyl-3-penten-2-one·H^+ 284 mμ).[7]

Solutions of Ia in 96% H_2SO_4 show little change in n.m.r. or ultraviolet spectra after one hour although decomposition is progressing. The ion becomes increasingly unstable (chemically) as the % H_2SO_4 is reduced from 85 to 52%. At 25°, the half-life is 700 seconds in 71% acid and 30 seconds in 52% acid. Extrapolations of the first order rate plots suggest that IIa is completely converted to Ia even in 52% acid. The mode of decomposition appears to be nucleophilic attack on the —CH_2—

(1) Grateful acknowledgment is made of partial support of this Research by grants from the Petroleum Research Fund of the American Chemical Society, and the National Science Foundation.

(2) N. C. Deno, H. G. Richey, Jr., J. D. Hodge, and M. J. Wisotsky, *J. Am. Chem. Soc.*, **84**, 1498 (1962).

(3) H. Hart and J. M. Sandri, *ibid.*, **81**, 320 (1959).

(4) The H_2SO_4 solution was held in plastic films between NaCl prisms. By taking two spectra, one with high clarity polyethylene and one with high clarity FEP fluorocarbon polymer (kindly supplied by the du Pont Company), the complete 2–15 μ region can be measured. The H_2SO_4 has a strong background absorption throughout this region, but the intense bands pierce through this background.

(5) IIa Solvolyzes at a moderate rate in acetic acid (and probably also methanol and ethanol) so that it was necessary to introduce IIa directly into 96% H_2SO_4 and dilute the resulting solutions.

(6) Simple protonated ketones do not absorb above 220 mμ (H. J. Campbell and J. T. Edward, *Can. J. Chem.*, **38**, 2109 (1960)).

(7) 4-Methyl-3-penten-2-one·H^+ has a strong absorption band at 1540 $cm.^{-1}$ in close agreement with the value 1533 $cm.^{-1}$ found in an alkenyl cation (ref. 2). This is ascribed to a stretching mode of the C···C···C system and suggests that the hydroxyalkyl cation form makes the dominant contribution to the structure of this protonated ketone. A second intense band at 1595 $cm.^{-1}$ may be the second stretching band of the allylic system though a band at 1590–1610 has been found in several simple protonated ketones including acetone·H^+.

of Ia to give ring opening analogous to the reaction of IIa in dilute acids observed by Hart and Sandri.[3] The decomposition product is a new ion (λ_{max} 285 mμ, $\epsilon > 3000$), half-formed above 80% H_2SO_4, and tentatively assigned structure Ie. Addition of solutions of Ie to water gives a compound whose b.p. and n.m.r. and infrared spectra are in accord with structure III. The addition of III to H_2SO_4 regenerates Ie.

The identification of Ib is less complete. The ultraviolet spectrum (λ_{max} 293 mμ, $\epsilon > 2600$)[8] is similar to that of Ia and Ie. Ib is half formed above 80% acid as is Ie. Immediate drowning of cold solutions of Ib in ice–10% NaOH gave a 25% yield of polymer and a 20% yield of an olefin which has a b.p. and n.m.r. and infrared spectra in agreement with the alkene (1,1-dicyclopropyl-2-methylpropene) derived from IIb by direct dehydration. In addition to decomposition by polymerization, Ib (or the alkene) is oxidized by 96% H_2SO_4 as evidenced by liberation of SO_2 and rising absorption around 300 mμ.

A number of C_4 and C_8 alkenes and alkanols form a species (possibly several species) in H_2SO_4 characterized by a broad absorption band around 293 mμ.[9] Its absorption and its thermodynamic stability (half-formed in 65% H_2SO_4)[9] are characteristic of alkenyl cations.[2] The slow formation from *t*-butyl alcohol,[10] the same rate of formation from *t*-butyl alcohol and 2-butanol,[9] and our observation that the kinetics of formation are not simply first order identify the 293 mμ species as an alkenyl cation rather than the *t*-butyl cation.[9]

(8) The ϵ values for Ib and Ie are for 96% H_2SO_4 solutions. The ions are not completely formed from their alkenes at these acidities.

(9) J. Rosenbaum and M. C. R. Symons, *Mol. Phys.*, **3**, 205 (1960).

(10) The 293 mμ species is reported to require two hours to half form from a $\sim 10^{-4}$ molar solution of *t*-butyl alcohol in 80 and 98% H_2SO_4 (ref. 9). In our experience with over a hundred carbonium ions including the aliphatic carbonium ions in this communication and ref. 2, the alcohol–carbonium ion equilibria were established within seconds.

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RECEIVED MARCH 23, 1962

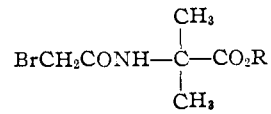
MODIFICATION OF A METHIONINE RESIDUE NEAR THE ACTIVE SITE OF CHYMOTRYPSIN

Sir:

We wish to describe a new method for the selective chemical modification of enzymes, and its application to the pancreatic protease, chymotrypsin. The basic principle is to combine an enzyme with a bifunctional reagent, so designed that it becomes covalently bound to an amino acid side chain at the active site, and then, fixed in position, reacts with another amino acid residue in the vicinity. The reaction of chymotrypsin with nitrophenyl esters,[1] during which the acyl group of the ester becomes attached to the hydroxyl group of the serine residue at the active site,[2] is a good one to demonstrate the utility of the method.

(1) C. E. McDonald and A. K. Balls, *J. Biol. Chem.*, **227**, 727 (1957).

(2) R. A. Oosterbaan and M. E. Van Adrichem, *Biochim. Biophys. Acta*, **27**, 423 (1958).



I, R = *p*-nitrophenyl; II, R = H

When chymotrypsin (1.4 mg./ml.) is treated at pH 5 with a tenfold molar excess (dissolved in 10% by volume of ethanol) of *p*-nitrophenyl bromoacetyl-α-aminoisobutyrate (I), m.p. 149–150° (*Anal.* Calcd. for $C_{12}H_{13}O_5N_2Br$: C, 41.75; H, 3.80; N, 8.12. Found: C, 42.02; H, 3.80; N, 7.88), its activity toward tyrosine ethyl ester[3] slowly decreases: to 40% in 1 hr., to 22% in 3 hr. After overnight dialysis, the activity remains the same, which would be expected by analogy with pivalyl-chymotrypsin.[1] Unlike pivalyl-chymotrypsin, however, chymotrypsin inactivated by I does not regain activity on treatment with a pH 8 buffer containing glycerol.[2] Typical final activities are given in Table I.

TABLE I

INHIBITION OF CHYMOTRYPSIN BY I AT PH 5: EFFECT OF IPA[a] ON THE INHIBITION; PHOSPHORUS CONTENT OF INHIBITED CHYMOTRYPSINS AFTER REACTION WITH DFP

Ratio: I/CT	Ratio: IPA/CT	% Activity	Ratio: P/CT
10.0	0	30	...
10.0	5	35	...
10.0	25	42	...
10.0	100	60	...
0	...	100	1.04
2.5	...	77	...
5.0	...	39	1.05
10.0	...	23	1.04
20.0	...	22	1.03

[a] Abbreviations: IPA = 3-indolepropionic acid; DFP = diisopropylfluorophosphate; CT = chymotrypsin.

At pH 6, the decrease in activity takes place more rapidly to give about the same (30%) end activity, while at pH 7, after a quick drop in activity (to 40% in 1 min.), recovery occurs slowly (3 hr.) to give a product having about 75% activity.[4]

The irreversible inactivation occurs subsequent to a specific reaction at the active site since (a) the rate of the "burst" of nitrophenol during the formation of the acyl enzyme is retarded by the presence of the chymotrypsin inhibitor,[5] 3-indolepropionic acid, which inhibits the inactivation of the enzyme (Table I); (b) bromoacetyl-α-aminoisobutyric acid (II)[6] in a 100-fold molar excess does not cause any inactivation under the usual reaction conditions (4 hrs. at pH 5, overnight dialysis); and (c) methionine (see below) in diisopropylphosphoryl-chymotrypsin is not attacked by I (Table II). A random alkylation reaction is thus excluded.

Reincubation of inhibited chymotrypsin with the inhibitor I failed to reduce its activity further.

(3) G. W. Schwert and Y. Takenaka, *ibid.*, **16**, 570 (1955). The final percentage activity is roughly the same toward N-acetyl-L-tyrosine ethyl ester.

(4) The activities measured during the course of the reaction probably represent the combined activities of reversibly and irreversibly inhibited enzyme, while those measured after long standing (dialysis) are due to irreversibly inhibited chymotrypsin.

(5) H. Neurath, J. A. Gladner and G. De Maria, *J. Biol. Chem.*, **188**, 407 (1951).

(6) E. Abderhalden and E. Haase, *Fermentforschung*, **12**, 313 (1931).