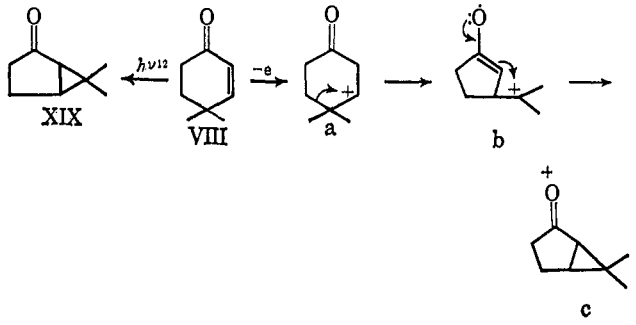
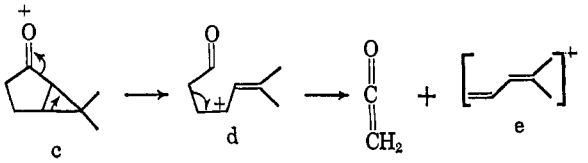
before fragmentation occurs in the vibronically excited state, a rearrangement of some of the molecular ions of VIII occurs to molecular ion c, formally analogous to that of compound XIX, the photoisomer derived from the enone VIII. One pathway by which



the ketene could be lost would be the subsequent fragmentation of this rearranged molecular ion c.



In the high-resolution mass spectrum of the bicyclic photoproduct XIX, the ion formed by elimination of ketene from the molecular ion makes the major contribution (% Σ_{39} 27.47) to the total ion current. The loss of C-2 and C-3 is confirmed in the spectrum of 6,6-dimethylbicyclo[3.1.0]hexan-2-one-3,3-d_2. It appears (see Figures 1 and 2) that the enone VIII generates a hydrocarbon fragmentation pattern primarily *via* loss of ketene and an oxygen fragmentation pattern by loss of ethylene. Decomposition of the bicyclic-[3.1.0] ketone XIX generates a strikingly similar hydrocarbon fragmentation pattern. Thus, 4,4-dimethyl-2-cyclohexenone may be another example in which rearrangement or fragmentation observed on photon absorption also occurs on electron impact.[13-16]

However, two points remain unexplained in this analogy to the photorearrangement. (a) Compounds I, VI, VII, and XIII, which do lose ketene on electron impact, have been observed not to rearrange on irradiation to the bicyclo system. (b) In addition, comparison of the mass spectrum of cholestenone's photoproduct[17] with that of cholestenone indicates that the "photorearrangement" cannot be the major path under electron impact leading to loss of ketene from cholestenone.

Nonetheless, the bicyclo[3.1.0] system remains an attractive and reasonable general intermediate from which to lose ketene. The bicyclo[3.1.0] system could be generated by any of several bond migrations in the cyclohexenone molecular ion, including the "photorearrangement" VIII → b. Migration to C-3 of a

(12) O. L. Chapman, T. A. Rettig, A. I. Dutton, and P. Fitton, *Tetrahedron Letters*, 2049 (1963).
(13) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959).
(14) N. J. Turro, D. C. Neckers, P. A. Leermakers, D. Seldner, and P. D. Angelo, *J. Am. Chem. Soc.*, **87**, 4097 (1965).
(15) P. Brown, J. Kossanyi, and C. Djerassi, *Tetrahedron Suppl.*, [I] **8**, 241 (1966).
(16) After submission of this manuscript, Professor Carl Djerassi of Stanford University informed us that similar observations had been recorded in his laboratory but with a different interpretation. See R. L. N. Harris, F. Komitsky, Jr., and C. Djerassi, *J. Am. Chem. Soc.*, in press.
(17) B. A. Shoulders, W. W. Kwie, W. Klyne, and P. D. Gardner, *Tetrahedron*, **21**, 2973 (1965).

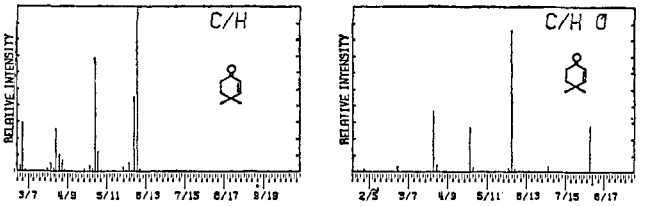


Figure 1. The complete high-resolution mass spectrum of 4,4-dimethylcyclohex-2-enone.

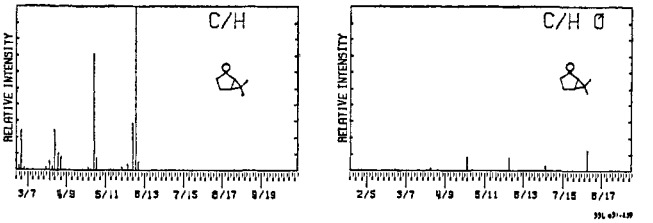
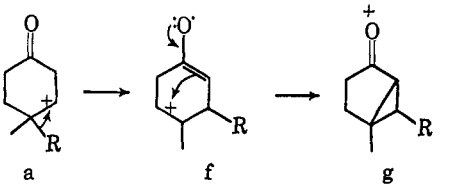


Figure 2. The complete high-resolution mass spectrum of 7,7-dimethylbicyclo[3.1.0]hexan-2-one.

methyl group from C-4[16] as shown in a → f could lead to formation of the ionized bicyclo[3.1.0]hexane-2-one derivative (f → g) in a manner analogous to that postulated in b → c. In polycyclic systems three different alkyl migrations are possible from the quaternary center γ to the carbonyl group. In VII and XIII



migration of the tertiary γ-hydrogen atom could also lead to a bicyclo[3.1.0]hexan-2-one (a → g). The suggestion is made, then, that ketene is lost from cyclohexenone derivatives through a rearranged bicyclo-[4.1.0]hexan-2-one system, whose formation requires one or another bond migration from the substituted γ carbon.

The structural features reported above are necessary for the loss of ketene from conjugated cyclohexenones, although reports indicate that the relative importance of this elimination may be altered by the presence of additional functional groups[18] and, in the rigid steroid systems, by different stereochemistry.[19]

Acknowledgment. We wish to thank Mr. B. Simoneit and Mr. D. H. Smith for determination of the high-resolution mass spectral data.

(18) S. H. Eggers, *Tetrahedron Letters*, 733 (1965).
(19) H. Egger, *Monatsh.*, **97**, 1290 (1966).
(20) (a) Hoffmann-LaRoche Company, Basel, Switzerland; (b) Public Health Service Predoctoral Fellow, 1965–1967.

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Intramolecular Migration of Tritium and Deuterium during Nonenzymatic Aromatic Hydroxylation

Sir:

It has recently been discovered that during enzymatic hydroxylation of aromatic substrates the substituent

Table I. The Absence of Intramolecular Migration of Deuterium during Electrophilic Aromatic Substitutions of Selectively Deuterated Substrates[a]

Compound	Reagent	Product
[*p*-^{2}H]Acetanilide (1.0)	Br_2–HOAc[b]	*p*-Bromoacetanilide (0.00)
[*p*-^{2}H]Acetanilide (1.0)	$(C_6H_5)_2CHOH$–trifluoroacetic acid	*p*-Acetamidophenyldiphenylmethane (0.03)
[*p*-^{2}H]Acetanilide (1.0)	HNO_3–H_2SO_4	Aromatic ring hydrogens exchange with solvent at room temperature
[*p*-^{2}H]Toluene (0.73)	Br_2	*p*-Bromotoluene (0.00)
[*p*-^{2}H]Toluene (0.73)	$(C_6H_5)_2CHOH$–trifluoroacetic acid	*p*-Tolyldiphenylmethane (0.01)
[*p*-^{2}H]Toluene (0.73)	Succinic anhydride–$AlCl_3$	$AlCl_3$ catalyzes the exchange of the ring protons with the proton of the carboxylic acid[c] produced in the reaction
[5-^{2}H]-Salicylic acid (0.38)	Br_2–HOAc	5-Bromosalicylic acid (0.02)
[*p*-^{2}H]N,N-Dimethylaniline (1.0)	Diazotized anthranilic acid	Methyl red (0.02)
[*p*-^{2}H]Phenol (0.44)	Cold, dilute H_2SO_4 and HNO_3	*p*-Nitrophenol (0.00)

[a] The numbers in parentheses indicate the number of deuterium atoms per molecule. [b] With the *para*-tritio compound, no retention was obtained with brominations in HOAc, pyridine, 8% HBr in HOAc, water, or the solid state. [c] This result is in agreement with the observations of J. Eastham, J. Bloomer, and F. Hudson, *Tetrahedron*, **18**, 653 (1962).

Table II. Nonenzymatic Hydroxylations

Oxidizing system	Substrate	Product	% retention of heavy isotope
$F_3CCOOOH$[a]	[*p*-^{3}H]Acetanilide	[*m*-^{3}H]*p*-Hydroxyacetanilide	9.6[e]
	[*p*-^{2}H]Acetanilide	[*m*-^{2}H]*p*-Hydroxyacetanilide	7.5
Fe(II), H_2O_2, EDTA[b]	[*p*-^{3}H]Acetanilide	*p*-Hydroxyacetanilide	1.9
Fe(II), O_2, ascorbic acid, EDTA[b]	[*p*-^{3}H]Acetanilide	*p*-Hydroxyacetanilide	1.0–1.2
Fe(III), H_2O_2, catechol[c]	[*p*-^{3}H]Acetanilide	*p*-Hydroxyacetanilide	0.9–1.0
Fe(II), O_2 ascorbic acid, EDTA[b,d]	[5-^{3}H]Salicylic acid	2,5-Dihydroxybenzoic acid	2.1
Elbs persulfate oxidation	[5-^{3}H]Salicylic acid	2,5-Dihydroxybenzoic acid	2.1

[a] Isotopically labeled acetanilides had less than 1% random label. The reaction procedure consists of storing a mixture of 1 ml of H_2O_2 (90%), 1 ml of trifluoroacetic acid, 0.5 ml of chloroform, and 1 mmole of acetanilide at 5° for 5 hr. Dilution of the reaction mixture with water, extraction with ethyl acetate, and paper chromatography of the residue left after evaporation of the solvent produced 1–2% yields of *p*-hydroxyacetanilide. The only other detectable product was *o*-hydroxyacetanilide. [b] Hydroxylated according to B. Brodie, J. Axelrod, P. Shore, and S. Udenfriend, *J. Biol. Chem.*, **208**, 741 (1954). Acetanilide underwent substantial *meta* hydroxylation. Salicylic acid formed both 2,3- and 2,5-dihydroxybenzoic acid. [c] H. Hamilton, J. Hanifin, and J. Friedman, *J. Am. Chem. Soc.*, **88**, 5266 (1966). [d] The random label in this substrate is approximately 2%. Microsomal hydroxylation of this substrate did not lead to significant retention (J. Daly, unpublished results). [e] This is the value obtained after the addition of cold carrier and recrystallization to constant specific activity.

(^{2}H, ^{3}H, Cl, Br, etc.) displaced by the entering hydroxyl migrates to an adjacent position in the aromatic ring.[1-6]

We now wish to report that this novel intramolecular shift occurs also in certain nonenzymatic substitution reactions. Because cationoid intermediates might be expected to facilitate such migrations, a variety of typical electrophilic aromatic substitution reactions were examined. Selectively deuterated and tritiated compounds were prepared either by palladium-catalyzed hydrogenolysis of the corresponding chloro, bromo, or benzoxazolyl ether[7] derivative or, in a few cases, such as the preparation of [*p*-^{2}H] toluene, by neutralization of organolithium compounds with deuterium oxide (Table I). Hydrogenolysis of *p*-O-tosyloxyacetanilide with Raney nickel in the presence of deuterium or tritium led to acetanilide with substantial randomization of label throughout the ring.

The position of the label in the deuterated compounds was ascertained by nmr spectroscopy and the extent of deuterium substitution measured by mass spectrometry. The products of electrophilic substitution from the specifically labeled compounds (Table I) showed no significant retention of deuterium, in agreement with earlier findings on bromination and nitration.[8]

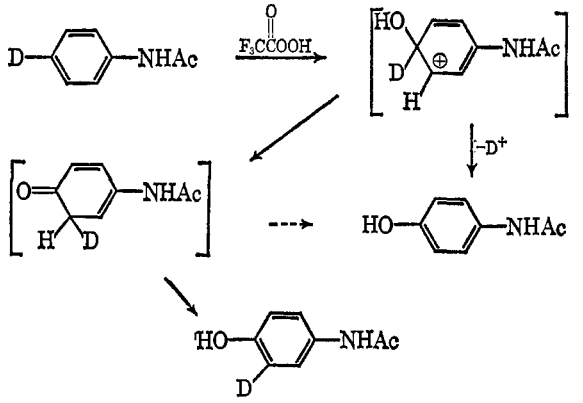
Of the six nonenzymatic hydroxylating systems examined (Table II), only hydroxylation of [p-^{3}H]- or [p-^{2}H]acetanilide by trifluoroperacetic acid led to significant migration rather than complete displacement of the para substituent.[9]

Unreacted starting material which was reisolated after the reaction retained the same specific activity and the label was still in the *para* position, since it was lost completely on bromination. The product, [*m*-^{2}H]*p*-hydroxyacetanilide, had deuterium in the ring

(1) G. Guroff, C. Reifsnyder, and J. Daly, *Biochem. Biophys. Res. Commun.*, **24**, 720 (1966).
(2) G. Guroff, M. Levitt, J. Daly, and S. Udenfriend, *ibid.*, **25**, 253 (1966).
(3) G. Guroff, K. Kondo, and J. Daly, *ibid.*, **25**, 622 (1966).
(4) J. Renson, J. Daly, H. Weissbach, B. Witkop, and S. Udenfriend, *ibid.*, **25**, 504 (1966).
(5) S. Udenfriend, P. Zaltzman-Nirenberg, J. Daly, G. Guroff, C. Chidsey, and B. Witkop, *Arch. Biochem. Biophys.*, in press.
(6) G. Guroff, J. Daly, D. Jerina, J. Renson, S. Udenfriend, and B. Witkop, *Science*, in press.
(7) W. J. Musliner and J. W. Gates, Jr., *J. Am. Chem. Soc.*, **88**, 4271 (1966).
(8) L. Melander, *Arkiv Kemi*, **2**, 211 (1950).
(9) The migration of a methyl substituent during hydroxylation of prehnitene with trifluoroperacetic acid and boron trifluoride in principle provides a precedent and analogy: C. Buehler and H. Hart, *J. Am. Chem. Soc.*, **85**, 2177 (1963).

positions adjacent to the hydroxyl group by comparison of the intensity of the nmr signals of the 3,5-ring protons with those of the 2,6 protons. The retention of tritium (9.6%) and deuterium (7.5%) obtained on *para*-hydroxylation of acetanilide with trifluoroacetic acid may be compared to the retention of tritium (40–50%) and deuterium (15%) in the enzymatic hydroxylation of acetanilide.[5]

Mechanistically, the hydroxylation with trifluoroperacetic acid is likely to involve attack by "OH^+" or a related species and presumably proceeds by the pathway



The nature of the oxygenating species involved in the enzymatic hydroxylation of aromatic substrates is still a subject for speculation. The hydroxylating systems of Table II have served as models for the hydroxylases.[10] The degree of retention in other model systems currently under investigation may serve as a useful further guide for the correlation of enzymatic and nonenzymatic hydroxylations.[11]

(10) V. Ullrich and H. Staudinger, in "Biological and Chemical Aspects of Oxygenases," K. Bloch and O. Hayaishi, Ed., Maruzen Co., Ltd., Tokyo, 1966, pp 235–249.

(11) A related phenomenon, the oxidative coupling of 4-^{3}H-2,6-xylenols, gives *para*-substituted polymers with 23% retention of tritium. In this case a radical coupling mechanism has been proposed in which electron-transfer resonance stabilization leads to (rearranged) phenonium ions: W. A. Butte, Jr., and C. C. Price, *J. Am. Chem. Soc.*, **84**, 3567 (1962).

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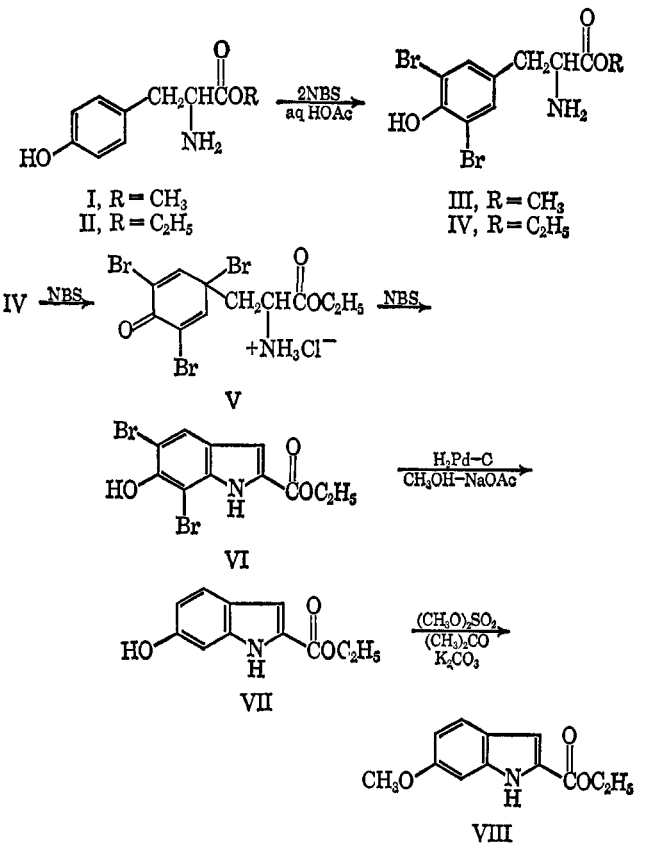
Chemical Conversion of Tyrosine to 6-Hydroxyindoles

Sir:

Silver oxide, ferricyanide, and other oxidants convert 3,4-dihydroxyphenylalanine (DOPA), 3-hydroxytyramine, noradrenaline, and related catecholamines to 5,6-dihydroxyindole derivatives.[1] The transformation of tyrosine to 5,6-dihydroxyindole-2-carboxylic acid, however, is only known as an enzymatic reaction and requires *tyrosinase* to effect the initial oxidation to DOPA.[2]

We wish to report the first direct nonenzymatic conversion of methyl or ethyl tyrosinate (I and II, respectively) to the monohydroxyindole VI by the action of



N-bromosuccinimide (NBS). This transformation was discovered when we observed that amino-terminal tyrosine peptides failed to undergo the expected cleavage on treatment with NBS.[3]

When 1.0 m*M* solutions of the tyrosine esters I–IV in 0.25 *M* HOAc were treated with aliquots of a 20 m*M* aqueous NBS solution, the rapid appearance of the characteristic bromo dienone chromophore ($\epsilon_{260-270}$ ~8000) was observed. This absorption reached a maximum with 3 equiv of NBS on I or II and 1 equiv on the dibromo derivatives III and IV. The bromo dienone V was isolated on a preparative scale from II and from the dipeptides Tyr-Ala and Tyr-Phe. The reactive bromine in V liberated iodine in the starch–potassium iodide test. The analysis accounts for the introduction of three atoms of bromine to give the tribromo dienone hydrochloride V. This remarkable intermediate, which has been discussed previously in connection with the mechanism of cleavage,[4] is apparently stabilized by the ionized amino group in close proximity to the dienone system. In such a labile system, intramolecular Michael addition of the amino group to the dienone would be competitive with the formation of the spirodienone lactone, the reaction underlying the cleavage of peptide bonds.

This was indeed found to be the case. In contrast to the comparatively stable bromo dienone lactone of the tyrosine peptide cleavage,[4] the absorption of the labile dienone V at 260 mμ on standing decreased slowly, and a new absorption at 320 mμ began to develop. This absorption reached a maximum with 2 equiv of NBS on esters III and IV and 4 equiv on esters I and II after approximately 16 hr at room temperature or

(1) W. L. Dulière and H. S. Raper, *Biochem. J.*, **24**, 239 (1930); *cf.* R. A. Heacock, *Chem. Rev.*, **59**, 181 (1959).

(2) H. S. Raper, *Biochem. J.*, **21**, 89 (1927).

(3) M. Wilchek and B. Witkop, *Biochem. Biophys. Res. Commun.*, **26**, 296 (1967).

(4) G. L. Schmir, L. A. Cohen, and B. Witkop, *J. Am. Chem. Soc.*, **81**, 2228 (1959).