take to 75% of the coenzyme Q_{10} supplemented control. The solanesyl, phytyl, dihydrophytyl, and nonadecyl analogs depressed enzyme activity to 40–60% of that of the supplemented control value; this was 5–15% below the activity observed in the unsupplemented controls.

HPB (100 mμmoles) also inhibited the intact DPNH-oxidase system in the absence of supplemental Q_{10} (Table III). In this case, the specific activity is intermediate between that observed when the system is treated with 100 and 200 mμmoles of HPB and 100 mμmoles of Q_{10}.

In both the extracted and intact DPNH-oxidase systems (Table III), an inverse relationship between enzyme activity and HPB concentration is observed. In the intact DPNH-oxidase system, the oxidase activity was depressed to 70, 40, and 10% of that of the supplemented control when 50, 100, and 200 mμmoles of HPB and 100 mμmoles of CoQ_{10} were added to the system. When the coenzyme Q_{10} content was increased from 100 to 200 mμmoles in the presence of 50 mμmoles of HPB, a partial reversal of the inhibition was observed. The oxygen uptake was elevated from 70 to 85% of the Q_{10}-supplemented control.

In the DPNH-oxidase system which had been extracted to remove Q_{10}, 200 mμmoles of HPB in the presence of 100 mμmoles of coenzyme Q_{10} completely inhibited the enzyme activity. As the concentration of the HPB was decreased to 100 and 50 mμmoles, keeping the supplement of CoQ_{10} constant, an increase in enzyme activity to 45 and 70% of the supplemented control was observed (Table III).

In both the succinoxidase and DPNH-oxidase systems of intact enzyme preparations, the 2,3-dimethoxy-5-hydroxy-6-phytyl-1,4-benzoquinone (HPB) was more active as an inhibitor than the other alkyl derivatives. Succinoxidase is more sensitive to HPB inhibition than DPNH oxidase. The structural nature of the aliphatic side chain in the 6 position seems to be less significant than the presence of the 5-hydroxy group for inhibitory activity. These exploratory data indicate that Q_{10} may reverse the inhibition by HPB of DPNH oxidase but may not reverse the inhibition of succinoxidase. This apparent difference is at least compatible with recent data showing two enzyme sites[8] for the participation of Q_{10} in electron transfer.

Acknowledgment. This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories, Rahway, N. J., and we express our appreciation to Dr. Max Tishler.

(8) G. Lenaz, G. D. Daves, Jr., and K. Folkers, *Arch. Biochem. Biophys.*, in press.

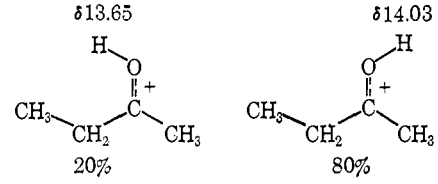
(9) Stanley G. Harris Postdoctoral Fellow for Biomedical Research.

Joseph C. Catlin, Ronald S. Pardini[9]
G. Doyle Daves, Jr., James C. Heidker, Karl Folkers
Stanford Research Institute
Menlo Park, California 94025
Received April 24, 1968

Protonated β-Phenyl Ketones. Intramolecular π Hydrogen Bonding[1]

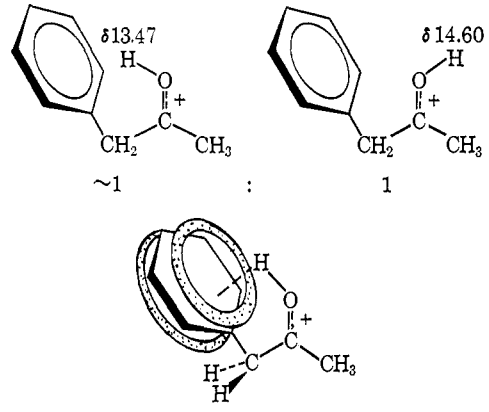
Sir:

In recent times protonated ketones have been observed by nmr in very strong acid media.[2] In such solvents under the right conditions, exchange of the C=O—H proton with the medium is sufficiently retarded that its nmr signal is observed. The chemical shift of the C=O—H proton can serve as a sensitive probe for inductive and conjugative electronic effects.[2] Further, with unsymmetrical ketones the C=O—H signals provide interesting information as to relative amounts of *syn* and *anti* isomers.[2] For example, in the case of protonated methyl ethyl ketone[2b] in SO_2–FSO_3H–SbF_5, the isomer ratio is 80:20, with the C=O—H proton *syn* to the smaller methyl group in the predominant isomer. In this connection protonated



β-phenyl ketones are of interest, and in this communication we report and discuss some of our observations on such species (Table I).

β-Phenyl ketones in an SO_2–FSO_3H–SbF_5 (9:7:2 mole ratio) medium at low temperatures typically show two C=O—H signals, one at unusually high field and the other at a much lower field. For example, phenyl acetone shows one signal at δ 13.47 ppm and the other at δ 14.60 ppm, for a Δδ of 1.13 ppm. As a result, the



chemical shift between *syn* and *anti* isomers is unusually high for β-phenyl ketones.[3] Further, the *syn*:*anti* ratios seem at first to be anomalous in view of the results obtained formerly in other cases. For example, with phenylacetone the isomer ratio is ~1:1, whereas on steric grounds it might have been expected to be much more lopsided than for methyl ethyl ketone.

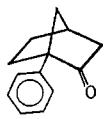
From the available evidence we can assign the high-field C=O—H signal of the protonated β-phenyl ketones to the *syn* (S) isomer with the proton *syn* to the phenyl group and thus shielded due to the magnetic anisotropy of neighboring phenyl. The low-field C=O—H signal is thus assigned to the *anti* (A) isomer. In this isomer the proton *anti* to the phenyl group is somewhat deshielded due to the latter's inductive effect. This inductive effect may be illustrated with 2-indanone,

(1) Research supported by the National Science Foundation.

(2) (a) T. Birchall and R. J. Gillespie, *Can. J. Chem.*, **43**, 1045 (1965); (b) M. Brookhart, G. C. Levy, and S. Winstein, *J. Am. Chem. Soc.*, **89**, 1735 (1967); (c) G. A. Olah, M. Calin, and D. H. O'Brien, *ibid.*, **89**, 3586 (1967).

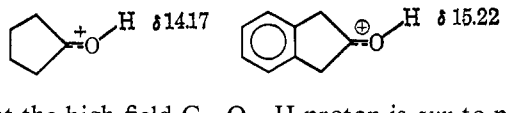
(3) Δδ is <0.4 ppm for all ketones not containing groups with large directional anisotropy.

Table I. Chemical Shifts of C=O—H Proton Signals for Some β-Phenyl Ketones

Ketone	Medium[a] SO_2–FSO_3H–SbF_5	Temp, °C	δ for C=O—H[b]	Rel amt, %
CH_3COCH_3	9:7:2	−59	14.24[2b]	
$C_6H_5CH_2COCH_3$ (I)	9:7:2	−58	S 13.47	56
			A 14.60	44
	0:5:1	−56	S 13.33	51
			A 14.65	49
$(C_6H_5)_2CHCOCH_3$ (II)	9:7:2	−60	S 13.85	39
			A 15.17	61
	0:5:1	−57	S 13.60	41
			A 15.17	59
$(C_6H_5CH_2)_2CO$ (III)	9:7:2	−58	S 13.85	100
p-$CH_3C_6H_4CH_2COCH_3$ (IV)	9:7:2	−55	Sulfination products	
	0:5:1	−55	S 13.33	59
			A 14.68	41
p-$NO_2C_6H_4CH_2COCH_3$ (V)	9:7:2	−58	A 15.12	100
$C_6H_5CH_2COCH_2CH_3$ (VI)	9:7:2	−56	S 13.60	86
			A 14.30	14
$C_6H_5CH_2COCH(CH_3)_2$ (VII)	9:7:2	−56	S 13.45	90
			A 14.25	10
 (VIII)	9:7:2	−65	S 13.37	29
			A 14.27	71

[a] *Ca.* 0.75 mmol of ketone in 0.3 ml of CH_2Cl_2 added to 0.7 ml of acid medium. [b] All chemical shifts (δ) are reported in parts per million downfield from TMS using internal CH_2Cl_2 (δ 5.30) as a secondary standard. These signals appear as singlets in these media at the indicated temperatures.

whose geometry precludes the large shielding effect due to the benzene ring.



That the high-field C=O—H proton is *syn* to phenyl may be proved in the case of phenylacetone by the spin–spin coupling patterns of the two C=O—H signals. In a 4:1:1 SO_2–FSO_3H–SbF_5 medium, the spectra are sufficiently well resolved that the high-field δ 13.47 ppm signal appears as a triplet with J = 1.1 cps, while the low-field δ 14.60 ppm signal appears as an incompletely resolved quartet.[4] Thus, the high-field signal corresponds to the *syn* proton with *cis* allylic-type coupling to two benzylic methylene protons while the low-field signal is due to the *anti* proton with *cis* allylic-type coupling to three methyl protons.

The "anomalous" *syn*:*anti* isomer ratios observed for phenylacetone as well as for the β-phenyl ketones II, IV, VI, and VII point to an attractive interaction between the *syn* C=O—H proton and the phenyl group which offsets the effect of its steric bulk. A weak hydrogen bond between the *syn* C=O—H proton and the π system of the benzene ring evidently serves to stabilize the *syn* isomer of the protonated β-phenyl ketones. Similar π hydrogen bonding has been called on in β-phenylethanols, *o*-phenylphenols, and other hydroxy compounds.[5] The usual method of study is infrared or ultraviolet spectroscopy. However, some work has been reported using nmr hydroxyl chemical shifts as a probe for these intramolecular effects.[5e] Oki, in his extensive series of papers, notes that the OH π bond is weaker than other hydrogen bonds, the energy of interaction being ~1–1.5 kcal/mol.[5c] The present indications are that the π hydrogen bonds in protonated β-phenyl ketones are of approximately the same strength.

The presence of a π hydrogen bond in the *syn* isomers of the protonated β-phenyl ketones is supported by the sensitivity of the *syn*:*anti* isomer ratio to phenyl substituent effects. The methyl group in *p*-methylphenylacetone (IV) causes a small but definite increase in the proportion of the *syn* isomer of the protonated ketone.[6] A large effect is observed due to the nitro group in *p*-nitrophenylacetone (V). With this compound the nitro group is protonated as well as the keto group (N=O—H resonance in 5:1 FSO_3H–SbF_5 at −70° is at δ 16.07 ppm),[7] and the benzene π system is made especially unfavorable for π hydrogen bonding. Thus, only one C=O—H signal, that for the *anti* isomer, is observed at δ 15.12 ppm; the *anti*:*syn* ratio in this case is, therefore, at least 20:1.

The β-phenyl ketones explored tend to be less stable in the strong acid media than alkyl or α-phenyl ketones. In SO_2-containing SO_2–FSO_3H–SbF_5 medium, ring sulfination is a competing reaction.[8] In the 9:7:2 medium with phenylacetone, sulfination is slight even after several days at −78°. However, sulfination proceeds at an appreciable rate at −50°. With *p*-methylphenylacetone, the methyl group is sufficiently activating to make sulfination the primary reaction in SO_2–FSO_3H–SbF_5. However, this ketone may be protonated in 5:1 FSO_3H–SbF_5. Ring sulfination

(4) The 9:7:2 medium is too viscous to resolve coupling with less than J = ~1.5 cps. A 4:1:1 medium similar to that used by Olah[2c] was required to resolve the small allylic-type coupling.

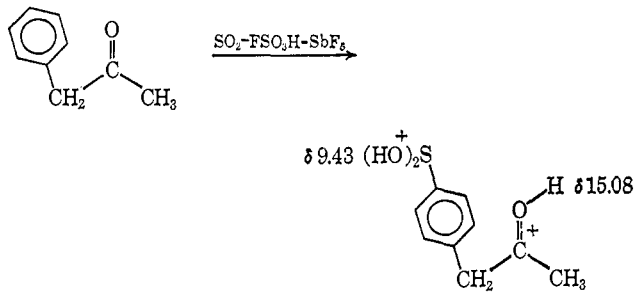
(5) (a) Review: M. Tichý, *Advan. Org. Chem.*, **5**, 115 (1965); (b) M. Oki, H. Iwamura, T. Onoda, and M. Iwamura, *Tetrahedron*, **24**, 1905 (1968); (c) M. Oki and H. Iwamura, *J. Am. Chem. Soc.*, **89**, 576 (1967), and earlier papers; (d) R. J. Piccolini, Ph.D. Thesis, University of California at Los Angeles, 1960; (e) D. C. Kleinfelter, *J. Am. Chem. Soc.*, **89**, 1734 (1967).

(6) The isomer ratio is somewhat medium dependent, due presumably to solvation energy differences. For compounds I and IV the isomer ratios are 51:49 and 59:41, respectively, in the 0:5:1 solvent.

(7) (a) Nitromesitylene reported in ref 2a, δ 14.86 ppm (−84°) for N=O—H; (b) H. Hogeveen, *Rec. Trav. Chim.*, **87**, 1320 (1967); nitrobenzene in HF–BF_3, δ_{NOH} 16.61 ppm (−100°).

(8) M. Brookhart, F. A. L. Anet, and S. Winstein, *J. Am. Chem. Soc.*, **88**, 5657 (1966).

makes itself evident by the appearance of a two-proton $S(OH)_2^+$ peak[8] for the protonated sulfinated ketone. With phenylacetone, which is partially sulfinated in the SO_2–FSO_3H–SbF_5 medium, the $S(OH)_2^+$ signal is at δ 9.43 ppm and a new very-low-field C=O—H signal is observed at δ 15.08 ppm. The latter signal has the same general appearance as does the incompletely resolved quartet for the C=O—H proton of the *anti* isomer of the protonated unsulfinated phenylacetone. The $S(OH)_2^+$ group is evidently sufficiently deactivating for π hydrogen bonding that the *anti* form is quite dominant over the *syn* isomer in the diprotonated sulfinated phenylacetone.



Above $-30°$, in media with or without SO_2, other modes of decomposition, leading to still unknown and possibly polymeric products, become significant with several of the β-phenyl ketones investigated.

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George C. Levy, S. Winstein
Contribution No. 2248, Department of Chemistry
University of California, Los Angeles, California 90024
Received April 22, 1968

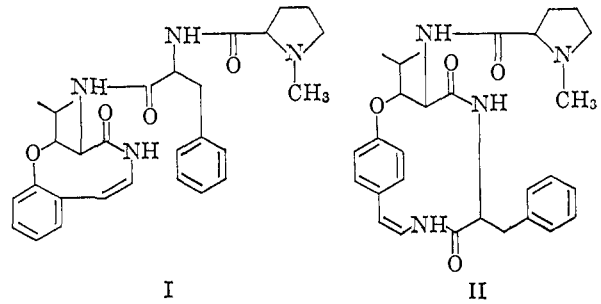
The Structure of Ceanothine-B

Sir:

Recent structure elucidation studies have dealt with the alkaloids of *Ceanothus americanus* root bark.[1,2] On the basis of chemical and spectroscopic data other workers assigned[2b] structure I to one of these alkaloids, ceanothine-B. Although much of their structure proof was convincing, the evidence for the *ortho*-fused oxazacyclononadiene ring and the amino acid sequence was equivocal. We now present data which establish a new structure of ceanothine-B.

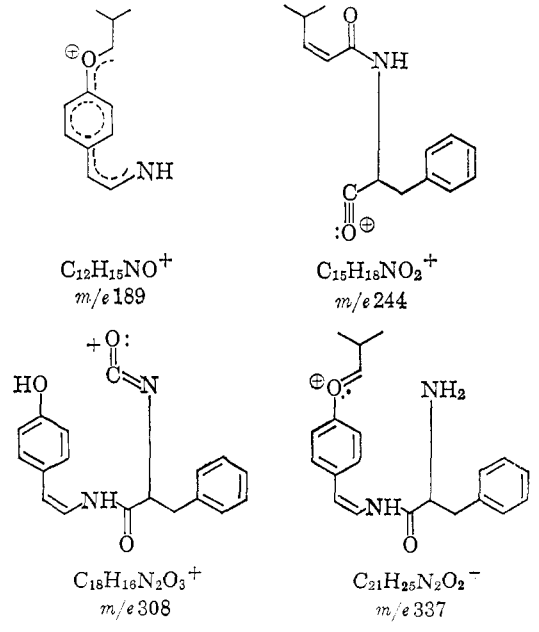
Our sample of ceanothine-B was obtained from dried, ground *C. americanus* root bark;[3] its properties, as well as those of dihydroceanothine-B, were in agreement with those reported.[2] High-resolution mass spectrometry and acid and alkaline hydrolysis followed by automatic amino acid analysis[4] of both ceanothine-B and dihydroceanothine-B confirmed the reported molecular formula, $C_{29}H_{36}N_4O_4$, and the presence of phenylalanine, β-oxygenated leucine, either an *o*- or a *p*-alkoxystyrylamine, and N-terminal N-methylproline.[5]

Ozonolysis of ceanothine-B followed by trimethyl phosphite decomposition of the ozonide and uv spectrophotometric analysis of the carbonyl products established that the alkoxy residue was fused *para* to the styryl double bond (λ_{max} 275 mμ; λ_{sh} 282, 290 mμ).[1] Since the seven bridging atoms of the *ortho* ring fusion of structure I are insufficient for *para* bridging, this observation required inclusion of the phenylalanine residue in the macrocycle and, consequently, attachment of the terminal N-methylproline residue to the leucine α-amino group. Revised structure II for ceanothine-B accommodates these requirements.



I II

Mass spectra of ceanothine-B and dihydroceanothine-B are completely and uniquely explicable on the basis of structure II and are irreconcilable with the amino acid sequence of structure I. Peaks at *m/e* 189, 244, 308, and 337 are consistent only with the sequence of structure II. High-resolution measurements of the latter three peaks verified the empirical formulas of the associated fragment ions.



Thus ceanothine-B is correctly represented by structure II. Moreover, our mass spectral and ozonolytic analyses of many different alkaloid fractions isolated from *C. americanus* root bark suggest strongly that among the constellation of alkaloid structures present

(1) F. K. Klein and H. Rapoport, *J. Am. Chem. Soc.*, **90**, 2398 (1968).
(2) (a) E. W. Warnhoff, S. K. Pradhan, and J. C. N. Ma, *Can. J. Chem.*, **43**, 2594 (1965); (b) E. W. Warnhoff, J. C. N. Ma, and P. Reynolds-Warnhoff, *J. Am. Chem. Soc.*, **87**, 4198 (1965).
(3) Plant material was furnished by Flint, Eaton and Co., Decatur, Ill. (now Baxter Laboratories, Morton Grove, Ill.).
(4) Automatic amino acid analyses were very generously conducted by Dr. Jonathan Dixon of the Hormone Research Laboratory, University of California Medical Center, San Francisco, Calif.
(5) Details of the methodology employed and the basis for these interpretations are given in ref 1.