solvation would not explain the failure of sulfur compounds to react. Owing to the attacking atom presumably being less heavily hydrogen bonded in the enzyme cleft than in water, sulfur derivatives should be favored over oxygen analogs (more energy must be spent in breaking water-oxygen hydrogen bonds). Also, although sulfur is larger than oxygen, it is not excluded from the reaction center, inferred from the recent report of lysozyme-catalyzed hydrolysis of a thiophenyl glycoside.[15]

In summary, the enzymic properties of lysozyme support the mechanism developed from the crystal structure. This is true as well for features of the mechanism not considered in this note, *e.g.*, cleavage of the C_1–O_4 bond, retention of configuration, and distortion of the substrate.[3]

Acknowledgment. I am grateful for a particularly stimulating discussion on carbon reactivity with Professors T. G. Traylor, C. L. Perrin, and J. W. Watson, and for comments made by Professors T. C. Bruice, T. H. Fife, W. P. Jencks, and L. B. Jones.

(15) G. Lowe, G. Sheppard, M. L. Sinnott, and A. Williams, *Biochem. J.*, **104**, 893 (1967).

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The Structure of Antheridiol, a Sex Hormone in *Achlya bisexualis*

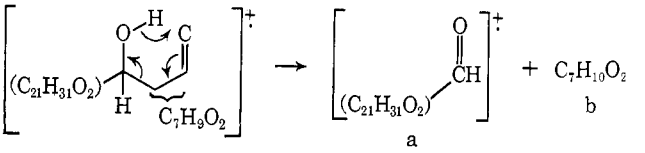
Sir:

Sexual reproduction in the aquatic fungus *Achlya bisexualis* is governed by specific substances.[1] One of these, designated antheridiol, is secreted by the female plant and brings about formation of antheridial hyphae on the male plant.[2] Antheridiol, which was isolated in crystalline form, is characterized by its melting point, 250–255° dec, a broad absorption band in the uv at 220 mμ (ε 1.7×10^4), strong absorption bands in the ir at 3390, 1742, and 1672 cm^{-1}, and extremely high and specific biological activity (at a concentration as low as 2×10^{-8} mg/ml).[2]

Because of the scarcity of material and the concomitant lack of any hint concerning the chemical nature of the hormone, a high-resolution mass spectrum was taken, using a few micrograms of the substance. The data, displayed in the form of an element map,[3] showed the elemental composition to be $C_{29}H_{42}O_5$. In addition, the distribution of the elemental composition of the fragment ions was very revealing. The ions of highest mass in the C–H, C–H–O, and C–H–O_2 groups were $C_{19}H_{23}$, $C_{21}H_{30}O$, and $C_{22}H_{30}O_2$, and the species $C_{19}H_{25}O$ and $C_{19}H_{27}O_2$ were particularly abundant. Such a distribution is strongly suggestive of a steroid containing two oxygen functions in the tetracyclic system. Assuming the abundant $C_{19}H_{27}O_2$ ion to originate by simple cleavage of the C-17,C-20 bond, the steroid nucleus would have to contain two double bonds.

At this point a steroidlike structure was assumed as a working hypothesis with the remaining ten carbons and three oxygens representing a "side chain" at C-17. The position of the third oxygen atom was revealed by the fact that the most intense peak in the spectrum corresponded to $C_{22}H_{32}O_3$, suggesting C-22 as the point of attachment. This is further confirmed by the scarcity of C–H–O_3 ions with less than 22 carbon atoms. The changes in elemental composition of the molecular ion and the fragment ions corresponding to $C_{19}H_{27}O_2$ and $C_{22}H_{32}O_3$ in antheridiol itself upon hydrogenation (30% Pd–C, in ethanol) and acetylation (acetic anhydride–pyridine), respectively, indicated that there are two carbon–carbon double bonds (M^+ of hydrogenation product $C_{29}H_{46}O_5$), one of which is in the ring system and one in the side chain beyond C-22, and two hydroxyl groups (M^+ of acetate $C_{33}H_{46}O_7$), again one in the ring system and one at C-22. In accord with the steroid hypothesis, it follows that there should be a carbonyl group as well as a hydroxyl and a carbon–carbon double bond in the tetracyclic system, while two oxygen functions, one carbon–carbon double bond, and two more double bonds or rings have to be within the last seven carbon atoms (C-23 through C-29).

Corroborating evidence was obtained from the ir spectrum of the tetrahydro derivative, which indicated that the carbonyl bands formerly at 1672 and 1742 cm^{-1} had shifted to 1709 and 1770 cm^{-1}. This is best accommodated by the presence of an α,β-unsaturated ketone (which would be in the steroid nucleus) and an α,β-unsaturated γ-lactone (located in the side chain). The high abundance as well as the hydrogen content of the $C_{22}H_{32}O_3$ ion (fragment a) requires that it is formed in a facile cleavage process involving the rearrangement of one hydrogen atom *away* from the C_{22} unit, the lactone carbonyl or the double bond functioning as the acceptor. The absence of this rearrangement after hydrogenation (the strong peak previously at *m/e* 344, $C_{22}H_{32}O_3$, appears at *m/e* 347, $C_{22}H_{35}O_3$) suggests a 1,3 relationship of C-22 hydroxyl and carbon–carbon double bond, as shown.



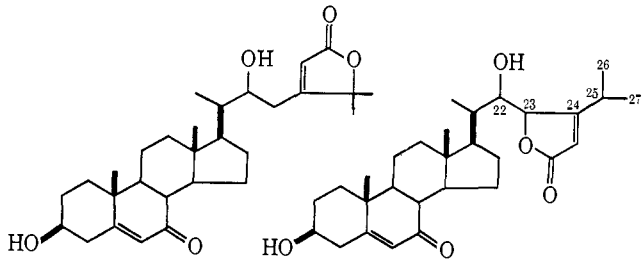
The positions of the hydroxyl and carbonyl groups in the tetracyclic system appeared to be at C-3 and C-7, respectively, on the basis of the mass spectrum of the ethylene ketal of tetrahydroantheridiol (1% toluenesulfonic acid in ethylene glycol, heated to 110° in toluene for 7 hr), which exhibited abundant ions of $C_5H_7O_2$ (*m/e* 99) and $C_7H_9O_3$ (*m/e* 141) in accordance with a 3-hydroxy-7-keto steroid.[4]

Dehydration of antheridiol (refluxed in methanol–hydrochloric acid) resulted in a product (M^+ at $C_{29}H_{40}O_4$) which contained only one oxygen in the tetracyclic system (abundant ionic species $C_{19}H_{25}O$ and $C_{22}H_{30}O_2$ but lack of $C_{19}H_{27}O_2$). This evidence combined with the change in the uv spectrum to λ_{max} 215 (ε 1.29×10^4) and 278 mμ (ε 2.37×10^4) suggested

(1) J. R. Raper, *Am. J. Bot.*, **26**, 639 (1939); J. R. Raper and A. J. Haagen-Smit, *J. Biol. Chem.*, **143**, 311 (1942).
(2) T. C. McMorris and A. W. Barksdale, *Nature*, **215**, 320 (1967).
(3) K. Biemann, P. Bommer, and D. M. Desiderio, *Tetrahedron Letters*, 1725 (1964).
(4) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, p 25.

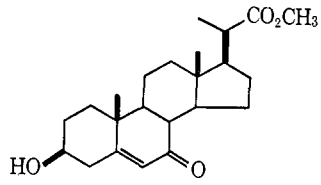
extended conjugation of the α,β-unsaturated ketone on dehydration to a $\Delta^{3,5}$-7-one.

At that point structures I and II appeared to be the most plausible ones which accommodate all the facts discussed above (the methyl group positions and the stereochemistry being based on biogenetic analogies). The facile rearrangement leading to the formation of fragment a would be sterically possible in the case of structure II since it could take place after opening of the lactone ring. The abundant $C_7H_{11}O_2$ ion (*m/e* 127) in the tetrahydro derivative favored II because in this



I II

case cleavage of the C-22,C-23 bond produces a positive charge next to oxygen rather than an unstabilized primary carbonium ion.



III

As a mass spectrometric model the ester III was prepared,[5] and the spectrum was found to correspond surprisingly close to that of antheridiol in the region below *m/e* 400, in terms of both abundance and elemental composition of the ions. The region between mass 300 and 400 reflected the presence of an additional CH_2O ($COOCH_3$ *vs.* CHO) in III as compared with fragment a. The few abundant low-mass ions present in the spectrum of antheridiol and absent in III are due to fragments (b and b $-$ CH_3) derived from the side chain, *i.e.*, *m/e* 126 ($C_7H_{10}O_2$) and 111 ($C_6H_7O_2$). The close similarity of the mass spectrum of antheridiol and compound III confirms the identity of the kind and position of substituents in the steroid nucleus of both compounds.

The nmr spectrum of antheridiol, determined (4 mg in $CDCl_3$–CD_3OD, 4:1) when more and purer material had become available, confirmed the choice of II for the structure of this compound. This spectrum shows two olefinic protons (at 5.69 and 5.77 ppm), the C-18 and C-19 methyl groups at 0.70 and 1.20 ppm, and C-26 and C-27 methyl groups (or *vice versa*) as doublets centered at 1.17 and 1.22 (spacing of 7 Hz). The C-21 methyl group is hidden under the complex multiplet caused by the C-19, C-26, and C-27 methyl groups. In addition the single proton on C-22 is a broad doublet at 3.60 ppm (specing of 8 Hz) coupled with the single proton on C-23 at 4.94 ppm (confirmed by spin-decoupling experiments).

(5) Prepared by CrO_3–HOAc oxidation at 60° of the methyl ester of 3β-acetoxybisnor-5-cholenic acid, followed by EtOH–K_2CO_3 hydrolysis; mp 184–186°, λ_{max} 237 mμ (ϵ 1.33 × 10^4), and ν_{max} 3570, 1724, and 1672 cm^{-1}.

In recent years, the isolation of a small number of hormones controlling sexual reproduction in fungi has been reported[6] and the characterization of one, sirenin, has just been accomplished.[7] Antheridiol is of particular interest since, if the proposed structure is correct, it is the first steroidal sex hormone to be recognized in the plant kingdom. It differs from mammalian sex hormones particularly in that it has a much longer side chain attached at C-17.

The proposed structure II for antheridiol must be considered as tentative until availability of this compound permits accumulation of further experimental evidence. The synthesis of structure II would be the most direct way to prove the structure of antheridiol since the isolation of this hormone in any quantity is difficult to achieve.

Acknowledgment. This investigation was supported by the National Institutes of Health, Grants GM 12150 and HD 00850 (at New York Botanical Garden) and GM 09352 (at Massachusetts Institute of Technology).

(6) L. Machlis in "The Fungi," Vol. II, G. C. Ainsworth and A. S. Sussman, Ed., Academic Press, New York, N. Y., 1966, p 415.

(7) L. Machlis, W. H. Nutting, and H. Rapoport, *J. Am. Chem. Soc.*, **90**, 1674 (1968).

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Gas-Phase Acidities of Carbon Acids

Sir:

Extensive studies of acidities of carbon acids have been reported for a number of solvent systems.[1] The problem of solvent medium effects makes interpretation difficult in some cases, so that it is desirable to undertake gas-phase studies not only to obtain intrinsic acidities but also to analyze solvent and counterion phenomena.[2] We wish to report some preliminary studies of gas-phase proton-transfer reactions of carbon acids and carbanionic bases, work which allows us to obtain relative gas-phase acidities. Making use of ion cyclotron resonance (ICR) spectroscopy, we have found the acidities: acetylacetone > acetyl cyanide > hydrogen cyanide. This work constitutes the first direct measurement of relative acidities of neutral compounds in the gas phase, and the technique is clearly applicable to studying a wide range of compounds.

The utility of ICR for studying ion–molecule reactions has been shown previously.[3] The detection of

(1) (a) C. D. Ritchie and R. E. Uschold, *J. Am. Chem. Soc.*, **90**, 2821 (1968), and references cited therein; (b) A. Streitwieser, Jr., J. H. Hammons, E. Ciuffarin, and J. I. Brauman, *ibid.*, **89**, 59 (1967); A. Streitwieser, Jr., E. Ciuffarin, and J. H. Hammons, *ibid.*, **89**, 63 (1967); (c) E. C. Steiner and J. M. Gilbert, *ibid.*, **85**, 3054 (1963); **87**, 382 (1965); E. C. Steiner and J. D. Starkey, *ibid.*, **89**, 2751 (1967); (d) K. Bowden and A. F. Cockerill, *Chem. Commun.*, 989 (1967).

(2) See ref 1a for relevant comments on this point.

(3) (a) L. R. Anders, J. L. Beauchamp, R. C. Dunbar, and J. D. Baldeschwieler, *J. Chem. Phys.*, **45**, 1062 (1966); (b) J. L. Beauchamp, L. R. Anders, and J. D. Baldeschwieler, *J. Am. Chem. Soc.*, **89**, 4569 (1967); (c) J. D. Baldeschwieler, *Science*, **159**, 263 (1968); (d) J. L. Beauchamp and S. E. Buttrill, Jr., *J. Chem. Phys.*, **48**, 1783 (1968); (e) J. M. Henis, *J. Am. Chem. Soc.*, **90**, 844 (1968); (f) G. A. Gray, *ibid.*, **90**, 2177 (1968).