The most precisely determined parameter is the C–C bond length at 1.532 Å. This is usually thought of as an average of two types of bonds, the shorter sp^2–sp^3 bonds to the carbonyl carbon and the normal C–C bond. Although these two bonds cannot be resolved it is interesting to consider their effect on the average. A commonly accepted value for a C–C bond adjacent to a carbonyl bond is 1.50 Å,[14] although there is some evidence in favor of a slightly longer bond.[15] Any value for the C–C bond adjacent to the carbonyl bond which is less than 1.51 Å would require a value of 1.538 Å or more for the average C–C bond length for the other C–C bonds. This value is larger than that normally found in hydrocarbons but it is in agreement with that found for *trans*-decalin by Davis and Hassel.[16] This may be due to a general expansion of bond lengths in the double ring or to a longer C–C bond at the bridgehead.

The bonded C–H and C–O distances overlap in such a way that in order to obtain reasonable results, a value had to be assumed for the amplitude of vibration of the C–H bond. The results obtained for the bond lengths using the assumed amplitude agree with those obtained from other molecules with a carbonyl bond.[17]

Acknowledgments. The author wishes to thank Dr. Lao-Sou Su for doing the photographic development, and the Indiana University Research Computing Center for the use of their CDC-3600 digital computer. Thanks are also due to Professor Russell A. Bonham for his guidance in the work.

(14) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," John Wiley and Sons, Inc., New York, N. Y., 1965, p 455.
(15) (a) A. Mossel and C. Romers, *Acta Cryst.*, 17, 1217 (1964); (b) R. Nelson and L. Pierce, *J. Mol. Spectry.*, 18, 344 (1965).
(16) M. I. Davis and O. Hassel, *Acta Chem. Scand.*, 18, 873 (1964).
(17) L. S. Bartell, J. P. Guillory, and A. T. Parks, *J. Phys. Chem.*, 69, 3043 (1965).

The Structure of Sirenin[1a]

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Contribution from the Departments of Chemistry and Botany, University of California, Berkeley, California 94720. *Received May 24, 1968*

Abstract: Sirenin (I) is the sperm attractant of the water mold *Allomyces*. Ozonolysis of its bis-NABS ester afforded NABS-hydroxyacetone in addition to two aldehydes (II and III), resulting from selective attack at each of the two double bonds. Structures for these aldehydes as well as for the dialdehyde IV obtained by treatment of sirenin with manganese dioxide were deduced from spectroscopic evidence. Oxidation of sirenin with periodate–permanganate led to the cyclopropane triester VI. Synthesis of this cyclopropane triester and three of its stereoisomers proved that sirenin was a sesquiterpene and possessed the carene bicyclic ring system. Additional comparison with *cis*- and *trans*-2-methyl-2-penten-1-ol allowed the stereochemistry and structure of sirenin to be established as 9-[(*E*)-4-hydroxy-3-methyl-2-butenyl]-10-hydroxy-2-carene.

The presence of hormones in the sexual reproduction of fungi was first suggested in 1880 but did not receive any experimental confirmation for another 40 years.[2] In 1958, the chemotactic hormone sirenin was first reported[3] and the presence of sexual hormones in other fungi has been demonstrated or postulated.[4] Our recent communication[5] provided the first complete structural characterization of a plant sex hormone, and a partial characterization of hormone A (antheridiol) has been reported.[6]

Sirenin is the sperm attractant produced by the female gametes of the water mold *Allomyces* and is active at concentrations of 10^{-10} *M*. The production, isolation, and characterization of sirenin and its 4-(4-nitrophenylazo)benzoate (NABS) esters have been described.[7] We now present evidence for the complete structure and stereochemistry of sirenin (I). It differs significantly from mammalian sex hormones in that sirenin is a sesquiterpene rather than a steroid.

Mass spectrometry and microanalyses[7] of sirenin and its derivatives established the molecular formula $C_{15}H_{24}O_2$ for sirenin. The formation of a diacetate and a bis-4-(4-nitrophenylazo)benzoate (NABS) ester (Ib) suggested that both oxygen functions in sirenin were alcohols which was further supported by ir absorption at 3600 cm^{-1}. That both hydroxyl groups were part of primary allylic alcohol moieties was indicated by the four-proton signal in the nmr spectrum at δ 3.97 which shifted to 4.75 in Ib. From the magnitude of the shifts of the vinyl proton signals at δ 5.38 and 5.80 in I to 5.59 and 5.98 in Ib, respectively, two primary allylic alcohols with a β-vinyl proton were postulated since the carbinol proton signal was unsplit. One tertiary and one vinyl methyl group in sirenin were also indicated by the singlets at δ 0.88 and 1.67, respectively.

In order to elucidate the nature of the double bonds, bis-NABS-sirenin (Ib) was partially ozonized. In

(1) (a) Support in part by the National Science Foundation is gratefully acknowledged; (b) Department of Chemistry; (c) Department of Botany.
(2) J. R. Raper, *Amer. J. Bot.*, 47, 794 (1960).
(3) L. Machlis, *Physiol. Plant.*, 11, 181 (1958).
(4) L. Machlis in "The Fungi," Vol. 2, G. C. Ainsworth and A. S. Sussman, Ed., Academic Press, New York, N. Y., 1966, Chapter 13.
(5) L. Machlis, W. H. Nutting, and H. Rapoport, *J. Amer. Chem. Soc.*, 90, 1674 (1968).
(6) T. C. McMorris and A. W. Barksdale, *Nature*, 215, 320 (1967).
(7) L. Machlis, W. H. Nutting, M. W. Williams, and H. Rapoport, *Biochemistry*, 5, 2147 (1966).

addition to recovered Ib, three new NABS esters were isolated by preparative tlc. The first compound was identified as the NABS ester of hydroxyacetone by comparison of the nmr, ir, R_f, melting point, and mixture melting point with those of an authentic sample. The second product, II, retained the nmr signals attributed to the tertiary methyl, one NABS-OCH_2C-(C)=C group, and the vinyl proton at 5.98. A two-proton "triplet" (J = 6.90 and 7.85 Hz for the low- and high-field portions, respectively) of doublets (J = 1.45 Hz) at δ 2.55 was shown to be coupled to a one-proton triplet at δ 9.79. Irradiation of the latter signal collapsed the doublets to singlets. These data were interpreted as resulting from loss of the hydroxyacetone-NABS ester portion from bis-NABS-sirenin (Ib) with concomitant formation of an aldehyde to yield II. A propionaldehyde residue attached to an asymmetric center would make the adjacent methylene protons nonequivalent[8] and cause unequal coupling to the methylene group α to the carbonyl. A 5-hydroxy-4-methyl-3-pentenyl moiety in sirenin accounts for these observations.

The remaining product (III) arises from cleavage of the other double bond since the vinyl proton signal at δ 5.98 is absent in III. Formation of a disubstituted acetaldehyde was shown by the diagnostic nmr and ir absorptions. The magnitude of the coupling constant (J = 4.00 Hz) for the aldehydic proton suggested a cycloproylcarboxaldehyde[9] which is further supported by the ir peak at 1694.[10] Appearance of a two-proton triplet (J = 6.35 and 6.60 Hz for the low- and high-field portions, respectively) at δ 2.53[11] and a two-proton singlet at δ 4.95 reflected the presence of a NABS-hydroxyacetone residue attached to a methylene group since the latter signal in III and its carbonyl peaks in the ir were identical with the corresponding absorptions in NABS-hydroxyacetone. Finally, the large downfield shift to δ 1.33 in III from 0.95 in II indicates the t-methyl is on the cyclopropane ring, *cis* to the newly formed aldehyde.[12] These data establish in sirenin a second trisubstituted double bond. It is contained in a ring; one carbon bears an ethylene and a hydroxymethyl group, while the other is attached to a proton and a secondary methylcyclopropane.

Confirmation of the β-cyclopropyl allylic alcohol was obtained when sirenin was treated with manganese dioxide[13] to yield the dialdehyde IV. In addition to the characteristic nmr and ir absorptions for an α,β-unsaturated aldehyde, the uv spectrum showed two distinct maxima at 232[14] and 260 nm. The latter is typical of β-cyclopropyl-α,β-unsaturated aldehydes or ketones,[15a] and the chromophores had the expected ratio of extinction coefficients.[14,15b]

The partial structures deduced from the ozonolysis data account for the carbon, oxygen, 23 of 24 hydrogens, and the four unsaturations (two rings and two double bonds) in sirenin. Structure I embodies all these features and accommodates the stereochemistry relevant to the cyclopropyl substituents.

Confirmation of 11 of the 12 remaining carbons (isolation of hydroxyacetone accounts for three carbons) was obtained by first oxidizing sirenin with periodate–permanganate[16] to yield the triacid V, characterized as its optically active trimethyl ester VI. In accord with the proposed structure, nmr spectroscopy established the presence of three methoxycarbonyl groups which was further supported by the formal loss[17] of one (m/e 254), two (m/e 222), and three (m/e 190) molecules of methanol in its mass spectrum. In order to establish the number of exchangeable protons and obtain information about the relative stereochemistry of the methyl and methoxycarbonyl groups on the cyclopropane ring, ester VI was treated with $NaOCH_3$–CH_3OD. Although the mass spectrum indicated four exchangeable protons, it was not possible to establish the presence of a d_5 compound[18] with certainty. Also the t-methyl signal at δ 1.15 did not shift.

By repeating the periodate–permanganate oxidation of sirenin in a deuterium medium, it was possible to show no deuterium incorporation and thus no epimerization had occurred in the reaction leading to ester VI. Consequently, the integrity of the stereochemistry in sirenin was maintained in the degradation ester VI. In obtaining this ester, an unexpected and selective esterification of acid V occurred in CD_3OD. Ester VII was characterized by a single methoxyl absorption corresponding to the signal at δ 3.58 in ester VI and by the formal loss[17] of one and two but not three molecules of CD_3OH. Thus two CD_3 groups had been incorporated into ester VII and are assigned to the propionate esters for the following reasons. At 60 MHz the two signals observed indicated two different kinds of methoxyl groups, the intensity of the signal at δ 3.60 being greater than the signal at 3.58. Secondly, it would be expected that the propionate esters are less hindered to nucleophilic attack than the cyclopropylcarboxylate and that the latter would be less reactive due to partial conjugation. In addition pK_a measurements[19] show that cyclopropanecarboxylic acid is slightly more acidic than propionic acid. It also appears that the cyclo-

(8) See M. L. Martin and G. J. Martin, *Bull. Soc. Chim. Fr.*, 2117 (1966). Dimethyl (+)-*trans*-homocaronate exhibits a multiplet for its methylene group: L. R. Subramanian and G. S. K. Rao, *Tetrahedron*, **23**, 4167 (1967).

(9) G. J. Karabatsos and N. Hsi, *J. Amer. Chem. Soc.*, **87**, 2864 (1965).

(10) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p 42.

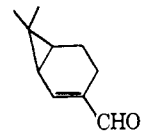
(11) The accuracy of determining the J value for the 2.53 triplet in III does not allow one to say definitely that the adjacent methylene protons are nonequivalent in contrast to the case in II. However, it is interesting to speculate that the C-8 methyl group is contributing more to the asymmetry at C-7 than the methine proton at C-6.

(12) Z. Arnold, *Chem. Commum.*, 299 (1967).

(13) F. Sondheimer, C. Amendolla, and G. Rosenkranz, *J. Amer. Chem. Soc.*, **75**, 5930 (1953); O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

(14) K. L. Stevens, R. E. Lundin, and R. Teranishi [*J. Org. Chem.*, 30, 1690 (1965)] report λ_{max}^{EtOH} 230 mμ (ϵ 13,000) for tetrahydrosinensal.

(15) (a) S. Julia, M. Julia, S-Y. Tchen, and P. Griffin, *Bull. Soc. Chim. Fr.*, 3207 (1964); (b) G. Buchi, W. Hofheinz, and J. V. Paukstelis [*J. Amer. Chem. Soc.*, **88**, 4113 (1966)] report λ_{max}^{EtOH} 263 mμ (ϵ 11,700) for



(16) E. von Rudloff, *Can. J. Chem.*, 43, 2660 (1965), and references therein.

(17) R. Ryhage and E. Stenhagen, "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, New York, N. Y., 1963, p 446.

(18) The apparent slow rate of exchange of the α proton in alkyl-substituted cyclopropylcarboxylates is in accord with the 0.7 D incorporated in the caryophyllene–ethyl diazoacetate adduct reported by E. W. Warnhoff and V. Dave, *Can. J. Chem.*, **44**, 621 (1966).

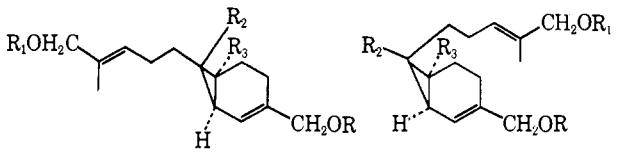
(19) "Constants of Organic Compounds," M. Kotake, Ed., Asakura Publishing Co., Ltd., Tokyo, 1963, pp 614–618.

propyl carboxyl can approach both of the remaining carboxyl groups for intramolecular stabilization of the transition state. Thus all factors are seen to be favorable for selective esterification.

Attempts to establish a cyclopropane ring in ester VI by the reaction of hydrogen chloride or hydrogen bromide in chloroform[20] led only to unchanged ester.[21]

Synthesis of triester VI and its three stereoisomers was best accomplished from the diester IX, obtained from esterification[22] of the known[23] *cis*-diacid VIII (Chart I). The copper-catalyzed[24] reaction with methyl

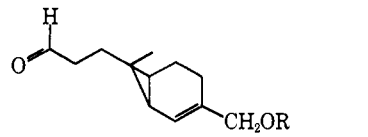
Chart I

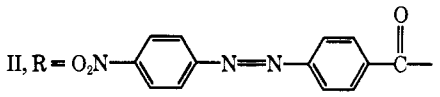


I, R = R_1 = R_3 = H; R_2 = CH_3 Ic, R = R_1 = R_3 = H; R_2 = CH_3

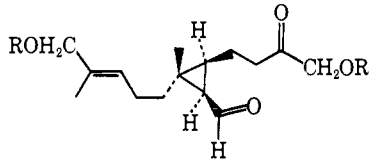
Ia, R = R_1 = R_2 = H; R_3 = CH_3 Id, R = R_1 = R_2 = H; R_3 = CH_3

Ib, R = R_1 = NABS; R_2 = CH_3; R_3 = H

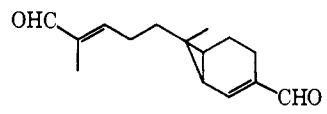




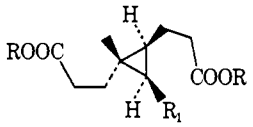
II, R = O_2N—N=N—



III



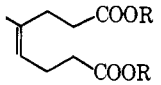
IV



V, R = H; R_1 = COOH

VI, R = CH_3; R_1 = $COOCH_3$

VII, R = CD_3; R_1 = $COOCH_3$



VIII, R = H

IX, R = CH_3

diazoacetate[25] converted the *cis*-diester IX into a mixture of the cyclopropane esters X and XI in the ratio of about 1:3. To obtain the two remaining stereoisomers of ester VI it was necessary to prepare the isomeric ester, *trans*-IX. Isomerization of *cis*-IX by sulfur dioxide,[26] *p*-toluenesulfinic acid,[27] or diphenyl disulfide[28] all led to the same mixture of isomers of IX in the ratio of about 2:1[29] by glpc. That the major component was the *trans* isomer was shown by nmr in which its vinyl methyl signal was located 3 Hz upfield[30] from the corresponding absorption in the *cis* isomer. Since the latter method produced the least impurities, it was used to obtain a mixture of the *cis* and *trans* isomers of ester IX. Attempts to acquire pure *trans*-IX by removal of the *cis* isomer with silver nitrate–silica gel, ammonical silver nitrate–silica gel tlc[31] and on a silver nitrate column were unsuccessful, while silver ion impregnated cation exchange resin[32] produced only partial separation. By employing preparative glpc, it was possible to obtain *trans*-IX (in about 98% purity) which was converted in an analogous fashion to the cyclopropane esters XII and XIII with the former predominating. Isolation by glpc afforded the pure ester XIII.

The stereospecificity[33] of the reaction places the propionate residues *cis* in esters X and XI and *trans* in XII and XIII. Application of nmr solvent shifts[34] to the cyclopropane esters X–XIII establishes the remaining stereochemistry. As shown in Table I, there is a relatively large (Δ = 0.31–0.36) upfield shift for the *t*-methyl signal in esters X and XII, while in the esters XI and XIII Δ is only 0.07–0.08. Since this behavior is well documented for methylcyclopropanes bearing a carbonyl function,[34] the methyl and methoxycarbonyl are *trans* in X and XII and *cis* in XI and XIII. These assignments are also in accord with predictions of the product distribution based upon stereochemical control of the reaction. Triester XIII was identical with the triester VI derived from sirenin by comparison of their nmr, ir, and mass spectra, and tlc and glpc (see Table I). Therefore XIII is *dl*-VI.

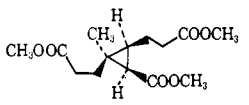
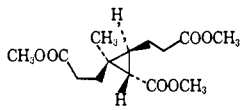
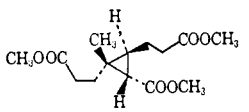
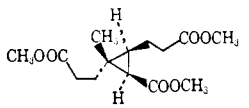
Consideration of the possible structures for sirenin[35] eliminates Ia, Ic, and Id which would yield cyclopropane esters with the *t*-methyl *trans* to the methoxycarbonyl. Since no epimerization occurred in obtaining ester VI, its stereochemistry is the same as sirenin's and these facts establish the bicyclic structure shown in I.

Evidence for the assignment of *trans* stereochemistry to sirenin's acyclic double bond followed from comparison with the nmr spectra of *cis*- and *trans*-2-methyl-2-penten-1-ol.[36] Vinyl proton absorption occurred at δ 5.25 and 5.40, respectively; sirenin exhibited a signal at δ 5.38. These data establish I uniquely as the structure of sirenin.

Sirenin belongs to the growing class of sesquiterpenes which are isoprene homologs of known monoterpenes.[37]

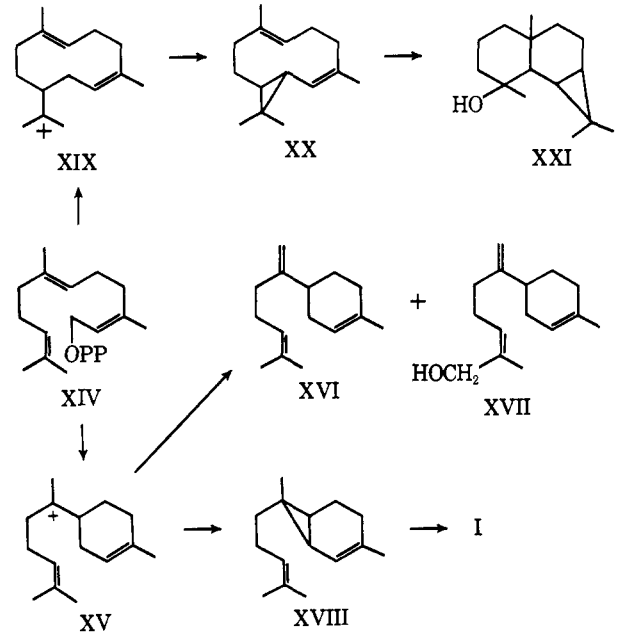
(20) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 2178 (1953).
(21) See reference cited in ref 18.
(22) F. H. Stodola, *J. Org. Chem.*, 29, 2490 (1964).
(23) E. Bertele, H. Boos, J. D. Dunitz, F. Elsinger, A. Eschenmoser, I. Felner, H. P. Gribi, H. Gschwend, E. F. Meyer, M. Pesaro, and R. Scheffold, *Angew. Chem.*, 76, 393 (1964).
(24) F. B. LaForge, W. A. Gersdorff, N. Green, and M. S. Schechter, *J. Org. Chem.*, 17, 381 (1952).
(25) E. B. Womack and A. B. Nelson, *Org. Syn.*, 24, 56 (1944).
(26) M. A. Golub, *J. Polym. Sci., Part B*, 4, 227 (1966).
(27) H. Nozaki, Y. Nisikawa, M. Kawanisi, and R. Noyori, *Tetrahedron*, 23, 2173 (1967).
(28) C. Moussebois and J. Dale, *J. Chem. Soc., C*, 260 (1966); E. J. Corey and E. Hamanaka, *J. Amer. Chem. Soc.*, 89, 2758 (1967).
(29) J. I. Cunneen, G. M. C. Higgins, and W. F. Watson [*J. Polym. Sci.*, 40, 1 (1959)] report 64% *trans*-3-methyl-2-pentene from the isomerization of either the *cis* or *trans* isomer.
(30) See R. B. Bates and D. M. Gale, *J. Amer. Chem. Soc.*, 82, 5749 (1960).
(31) R. Wood and F. Snyder, *J. Amer. Oil Chem. Soc.*, 43, 53 (1966); S. P. Dutta and A. K. Barua, *J. Chromatogr.*, 29, 263 (1967).
(32) E. A. Emken, C. R. Scholfield, and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, 41, 388 (1964); E. A. Emken, C. R. Scholfield, V. L. Davison, and E. N. Frankel, *ibid.*, 44, 373 (1967).
(33) See reference cited in ref 14.
(34) J. Ronayne and D. H. Williams, *J. Chem. Soc, C*, 2642 (1967), and references therein.
(35) Fusion of the cyclopropane and cyclohexene rings is assumed possible only with *cis* geometry.
(36) K. C. Chan, R. A. Jewell, W. H. Nutting, and H. Rapoport, *J. Org. Chem.*, 33, 3382 (1968).
(37) For a representative list, see I. C. Nigam and L. Levi, *J. Chromatog.*, 23, 217 (1966).

Table I. Nmr Chemical Shifts of the *t*-Methyl Group in Esters X, XI, XII, and XIII and Their Glpc Relative Retention Times (rrt)

Ester	δ^{CCl_4}	$\delta^{C_6H_6}$	rrt
 X	1.10	0.74	0.91
 XI	1.14	1.07	1.03
 XII	1.11	0.80	1.10
 XIII	1.15	1.07	1.00

It is the first[38] sesquiterpene which is an isoprenoid homolog of the terpene 2-carene. In analogy to the biogenesis of 2-carene, the cyclization of *cis*-farnesyl pyrophosphate (XIV, Scheme I) to the intermediate

Scheme I. Possible Biogenesis of Sirenin



XV, postulated to give β-bisabolene (XVI) and lanceol (XVII), can give rise to deoxysirenin (XVIII)[38] by a 1,3 deprotonation.[39] This mechanism is also postulated in the biogenesis of sesquiterpenes containing a *gem*-dimethylcyclopropane ring leading through the intermediates XIX and XX to maaliol (XXI).[39]

(38) While this manuscript was in preparation, Y. Ohta and Y. Hirose [*Tetrahedron Lett.*, 1251 (1968)] reported the structure of sesquicarene which is the same as the structure proposed for deoxysirenin (XVIII, Scheme I).

(39) W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.* (London), 21, 331 (1967).

The evidence presented for the structure of sesquicarene[38] indicates that it has the same stereochemistry as sirenin. Since the sign of the specific rotation[7] of sirenin is the same as sesquicarene's, sirenin is postulated to have the same absolute configuration, and this is depicted in I.

To our knowledge sirenin is only the third bicyclic sesquiterpenediol reported[40] and also the first sesquiterpene containing a vinylogous cyclopropylcarbinyl group with a fused cyclopropane ring, although two others are known in which there is a spirocyclopropane ring.[41]

Experimental Section[42]

Ozonolysis of Bis-NABS-sirenin (Ib). A 182-mg (0.25 mmol) sample of bis-NABS-sirenin (Ib) was dissolved in a solution of 100 ml of methylene chloride, 30 ml of chloroform, and 0.02 ml of pyridine and cooled at −60°. After about 0.3 mmol of ozone was absorbed by the solution during 1 hr, 2.0 ml (17 mmol) of trimethyl phosphite was added and the solution left at −40° for 16 hr. After evaporation, the crude product (236 mg) was applied to 60 g of silica gel and eluted with chloroform to yield 179 mg of NABS esters. Separation by preparative tlc afforded four major products. The R_f 0.55 spot was identified as recovered Ib (43 mg): δ ($CDCl_3$) 0.95 (3 H, s, tertiary CH_3), 1.78 (3 H, broadened s, C=CCH_3), 4.75 (4 H, s, two NABS-OCH_2C(C)=C), 5.59 (1 H, t, J = 7 Hz, C=C*H*CH_2), 5.98 (1 H, broadened s, C=CH(C)). The spot at R_f 0.26 afforded 29 mg and was identified as the NABS ester of hydroxyacetone: mp 167–168°; δ 2.27 (3 H, s, CH_3C=O), 4.95 (2 H, s, NABS-OCH_2C=O); $\nu_{max}^{CHCl_3}$ 1736 and 1723 by comparison with a sample obtained from the ozonolysis of the NABS ester of 2-methyl-2-propen-1-ol. The third substance (II) at R_f 0.38 amounted to 11 mg after an additional preparative tlc and had mp 115–117°; δ ($CDCl_3$) 0.92 (3 H, s, tertiary CH_3), 2.55 (2 H, "triplet" (J = 6.90 and 7.85 Hz for the low-field and high-field portions, respectively) of doublets, J = 1.45 Hz), 4.75 (2 H, s, one NABS-OCH_2C(C)=C), 5.97 (1 H, broadened s, C=CH(C)), 9.79 (1 H, t, J = 1.5 Hz); $\nu_{max}^{CHCl_3}$ 2736 and enhanced absorption at 1722; mol wt 447 (mass spectrum). The fourth product (III) isolated (R_f 0.07) amounted to 22 mg after preparative tlc and had δ ($CHCl_3$) 1.33 (3 H, s, tertiary CH_3), 1.77 (3 H, broadened s, C=CCH_3), 2.53 (2 H, t, J = 6.35 and 6.60 Hz for the low-field and high-field portions, respectively), 4.75 (2 H, s, one NABS-OCH_2C(C)=C), 4.95 (2 H, s, NABS-OCH_2C=O), 5.56 (1 H, t, J = 7 Hz, C=C*H*-CH_2), 9.65 (1 H, d, J = 4.00, CHC*H*O); $\nu_{max}^{CHCl_3}$ 2740, 1736, 1723, and 1694.

Oxidation of Sirenin (I) with Manganese Dioxide. Sirenin (R_f 0.0) had the following spectral properties: δ ($CDCl_3$) 0.88 (3 H, s, tertiary CH_3), 1.62 (2 H, broadened s, 2 OH, disappearance after treatment with D_2O), 1.67 (3 H, broadened s, C=CCH_3), 3.97 (4 H, s, 2 C=C(C)C*H*$_2$OH), 5.39 (1 H, t, J = 7 Hz, C=C*H*CH_2), 5.80 (1 H, broadened s, CH=C); $\nu_{max}^{CHCl_3}$ 3600. To 5 ml of benzene was added 12 mg (0.51 mmol) of sirenin (I) followed by 93 mg (1.07 mmol) of manganese dioxide. After shaking for 39 hr, the reaction mixture was centrifuged, the supernatant removed, and the residue rinsed with benzene. The combined benzene solutions were filtered and the filtrate was evaporated to give 8 mg (68%) of the dialdehyde IV: δ ($CDCl_3$) 0.94 (3 H, s, tertiary CH_3), 1.78 (3 H, broadened s, C=CCH_3), 6.48 (1 H, t, J = 7 Hz), 7.00 (1 H, d, $J \sim$ 3.5 Hz, CHC*H*=C), 9.41 (2 H, two signals separated by ~0.5 Hz); $\nu_{max}^{CHCl_3}$ 2710, 1686, 1672, and 1630; λ_{max}^{EtOH} 232, 260 nm ($\epsilon^{232}/\epsilon^{260}$ = 1.13); mol wt 232 (mass spectrum); R_f 0.33.

Oxidation of Sirenin (I) with Periodate–Permanganate. A. In Protium Medium. To a solution of 88.7 ml of 0.1 *M* sodium periodate (8.95 mmol), 20.5 ml of 0.01 *M* potassium permanganate

(40) Cryptomeridiol[39] and oplodiol (H. Minato and M. Ishikawa, *J. Chem. Soc., C*, 423 (1967)] are the two others.

(41) See illudin S and M.[39]

(42) All melting points are uncorrected. Microanalyses were performed by the Microchemical Laboratory, University of California. Nmr spectra were recorded on Varian A-60 or HA-100 spectrometers using internal TMS (δ 0). Ir spectra were recorded on Perkin-Elmer 237 or 421 spectrometers and are reported in reciprocal centimeters, cm^{-1}. Mass spectra were recorded on a Varian M66 or a CEC 103 spectrometer. Preparative and analytical thin layer chromatography was on silica gel, developed with chloroform.

(0.23 mmol), and 149.2 ml of 0.03 *M* potassium carbonate (4.48 mmol) was added a solution of 88 mg (0.37 mmol) of sirenin (I) in 50 ml of *t*-butyl alcohol and 150 ml of water followed by sufficient water to bring the volume to 500 ml. After 50 hr (pH 8.4), 7.8 mmol of periodate/mol of sirenin had been consumed, and the reaction was terminated by adding 3.75 g (28.9 mmol) of sodium arsenite and 10 g of sodium bicarbonate in 100 ml of water. After evaporation of the solution (pH 9.6), 25 ml of water was added and the pH adjusted to 2.9 with phosphoric acid. Sufficient sodium sulfite was added to prevent formation of iodine. Continuous ether extraction of the acidic solution for 80 hr yielded a total of 115.5 mg (dried over sodium sulfate) of viscous substance. Sequential rinsing with petroleum ether and methylene chloride left an ether-soluble residue which after evaporation yielded 54 mg (59%) of the triacid V. Subsequent treatment of V with diazomethane afforded the crude trimethyl ester VI which was chromatographed on 5 g of neutral alumina, activity III. Elution first with cyclohexane and then with benzene yielded 38 mg (60%) of the pure trimethyl ester from the latter as a mobile liquid: R_f 0.4; $[\alpha]^{22}D$ −20° (*c* 1.0, $CHCl_3$); $\nu_{max}^{CCl_4}$ 1744, 1736, 1720; δ (CCl_4) 1.15 (3 H, s, tertiary CH_3), 3.58, 3.60, 3.61 (9 H, s's, 3 OCH_3); δ (C_6H_6) 1.07 (3 H, s, tertiary CH_3), 3.33, 3.34, 3.36 (9 H, s's, 3 OCH_3); *m/e* (relative intensity) 254 (45), 222 (92), 190 (7), 163 (100).

B. In Deuterium Medium. To a solution of 737 mg (3.45 mmol) of sodium periodate, 14 mg (0.089 mmol) of potassium permanganate, and 239 mg (1.72 mmol) of potassium carbonate in 125 ml of deuterium oxide (99.9% D_2O) was added a solution of 35 mg (0.15 mmol) of sirenin (I) dissolved in 20 ml of *t*-butyl alcohol and 35 ml of deuterium oxide followed by sufficient D_2O to bring the volume to 200 ml. After 49 hr, 8.7 mmol of periodate/mol of sirenin had been consumed. To this solution (pH 8.7) was added 1.30 g (10.0 mmol) of sodium arsenite and 4.00 g of sodium bicarbonate in 40 ml of water and the resulting mixture (pH 9.3) evaporated, leaving a residue which was taken up in about 25 ml of water and the pH adjusted from 9.8 to 3.0 with phosphoric acid along with sufficient sodium sulfite to prevent iodine formation. The acidic solution was continuously extracted from ether, and the extract dried over sodium sulfate and then evaporated to give 39 mg of crude acid V which was rinsed with methylene chloride to finally give 27 mg (80%) of the acid V. Its nmr spectrum in methanol-d_4 was the same as the previous spectrum except for the reduction in a minor peak at δ 1.31.

Esterification[43] with diazomethane converted the acid to 17 mg (52%) of the ester VII after chromatography on alumina, $\nu_{max}^{CCl_4}$ 1743–1735; the nmr spectrum (CCl_4) was the same as the spectrum determined for ester VI except for the absence of the signals at δ 3.60 and 3.61; *m/e* (relative intensity) 260 (26), 257 (14), 225 (76), 222 (15), 190 (12), 163 (87), and 135 (100).

Hydrolysis of 17 mg (0.056 mmol) of the ester VII in 0.5 ml of dioxane was accomplished by adding a solution of 15 mg (0.36 mmol) of sodium in 0.5 ml of deuterium oxide. After heating at 95° (bath) for 21 hr, evaporation of the reaction mixture gave a residue which was dissolved in water and the pH adjusted from 10.4 to 3.1 with phosphoric acid. Continuous ether extraction produced an extract which was dried over sodium sulfate to yield, after evaporation, 14 mg of the crude acid V from which 12 mg (82%) of the acid V was obtained after rinsing with the methylene chloride.

Esterification with diazomethane converted the acid to 11.0 mg of the impure ester. Chromatography on alumina gave 10 mg (71%) of an ester which was identical with the ester VI by nmr and mass spectroscopy.

Deuterium Exchange of Ester VI. To 8 mg (0.028 mmol) of ester VI in 0.5 ml of methanol-d_1 was added 0.0056 mmol of sodium methoxide in 5.4 μl of methanol-d_1 solution and the reaction mixture heated for 24 hr at 80° (bath) in a sealed tube. The residue obtained from evaporation of the solvent was rinsed wih carbon tetrachloride and the solution evaporated to give 12 mg of yellow oil. Considerable exchange was indicated by nmr. Repetition of the procedure yielded 6 mg of exchanged ester. After chromatography on alumina (shown not to cause loss of deuterium from the α position of esters), 3.5 mg of purified ester was obtained which had δ (CCl_4) 1.27, 1.40, 1.48 (~2 H, all single peaks), 1.60 (2 H, broadened singlet), 1.9 (2 H, m), in addition to the same four methyl signals observed in ester VI; *m/e* (relative intensity) 258 (12), 257 (10), 226 (62), 194 (17), 137 (99), 95 (100).

(*Z*)-4-Methyl-4-octenedioic Acid (VIII). The literature synthesis,[23] which employed methyl esters, was applied[44] to the analogous ethyl esters to obtain the diacid VIII.

Dimethyl (*Z*)-4-Methyl-4-octenedioate (IX). The diacid VIII was esterified[22] in 97% yield to give the *cis* diester IX: bp 118–121° (5.5 mm); δ (CCl_4) 1.63 (3 H, broadened s, $C{=}CCH_3$), 2.19, 2.22, 2.26 (8 H, m, CH_2), 3.54 (6 H, s, OCH_3), 5.04 (1 H, poorly defined triplet, $C{=}CHCH_2$).

Anal. Calcd for $C_{11}H_{18}O_4$: C, 61.7; H, 8.5. Found: C, 61.9; H, 8.2.

Reaction of Dimethyl (*Z*)-4-Methyl-4-octenedioate (*cis*-XI) with Methyl Diazoacetate. To 3.00 g (0.014 mol) of diester IX and 5.5 g of copper bronze at 110–120° was added dropwise a solution of 5.00 g (0.023 mol) of IX in 33.0 g (0.33 mol) of methyl diazoacetate over 1.25 hr. After heating an additional 0.25 hr at 140–150°, the cooled reaction mixture was passed through a fritted glass filter and the filtrate distilled at 100° (~13 mm) to remove dimethyl fumarate and maleate. Chromatography of 4.8 g of the distilland on 250 g of Kiesel gel (for tlc)–chloroform yielded, among others, four fractions containing the *cis*-IX and the cyclopropane esters X and XI; the fraction number, milligrams, and per cent of *cis* diester IX and triesters X and XI are given, respectively, as follows: 1, 445, 83, 0; 2, 653, 36, 52; 3, 978, 0, 94; 4, 288, 0, 21. The composition of each fraction was determined by glpc (5% SE-30 at 210°) from which a total yield of 80% was calculated when corrected for recovered *cis*-IX. Preparative glpc (10% SE-30 at 210°) of fraction 3 yielded 52 mg of the triesters X and XI in the ratio of 1:3 with retention time 6 min: δ (CCl_4) 1.10 (s, tertiary CH_3), 1.14 (s, tertiary CH_3), 1.21–2.45 (m, CH, CH_2), 3.54, 3.55, 3.56, 3.57 (s's, OCH_3); δ (C_6H_6) 0.74 (s, tertiary CH_3), 1.07 (s, tertiary CH_3), 1.22–1.62 (m), 1.92–2.26 (m), 3.30, 3.32, 3.34 (s's, OCH_3); *m/e* (relative intensity) 254 (72), 223 (100), 222 (96), 190 (11), 163 (81), 135 (79).

Anal. Calcd for $C_{14}H_{22}O_6$: C, 58.7; H, 7.7. Found: C, 58.6; H, 7.8.

Dimethyl (*E*)-4-Methyl-4-octenedioate (*trans*-IX). Isomerization of 4.00 g (18.7 mmol) of *cis*-IX as a 25 m*M* solution in cyclohexane containing 4.08 g (18.7 mmol) of diphenyl disulfide afforded 7.87 g (97%) of a mobile yellow liquid. Chromatography on 120 g of 20% silver nitrate treated alumina[45] with benzene yielded 4.3 g of liquid which contained the *cis* diester retention time (25 min), the *trans* diester (28 min), and an unidentified component (29 min) in the ratio of 1:2:1.5 according to glpc (30% QF-1 at 200°). Preparative glpc yielded 390 mg of *trans*-IX of about 98% purity (glpc) and had δ (CCl_4) 1.60 (3 H, broadened s, $C{=}CCH_3$), 2.19, 2.23 (8 H, m, CH_2), 3.53 (6 H, s, OCH_3).

Reaction of Dimethyl (*E*)-4-Methyl-4-octenedioate with Methyl Diazoacetate. Using 390 mg (1.8 mmol) of *trans*-IX, 271 mg of copper bronze, and 2.16 g (21.6 mmol) of methyl diazoacetate, the reaction was carried out as described for the *cis* diester to give 1.25 g of crude reaction product. Chromatography on 20 g of Kiesel gel (chloroform) gave products in the second (370 mg) and third (335 mg) fractions in 42% yield (glpc: 5% SE-30 at 210°). Fraction 2 had singlets at δ (C_6H_6) 1.07 (major isomer) and 0.80 (minor isomer), while fraction 3 had only the signal at δ 0.80. Repetition of the chromatography of fraction 2 afforded tertiary fractions (100 mg) of impure triester XIII from which preparative glpc (10% QF-1 at 160°) yielded 9.0 mg of the pure triester XIII. Its nmr (CCl_4 and C_6H_6), ir, and mass spectra corresponded to the degradation ester VI as well as its retention time on glpc (see Table I) and R_f on tlc when cochromatographed.

(43) After sitting 2 days in methanol-d_4 at room temperature (nmr tube).

(44) Intermediates leading to *cis* ester IX were prepared by K. E. Opheim.

(45) R. Wolovsky, *J. Amer. Chem. Soc.*, 87, 3638 (1965).