Acknowledgment. The authors express their gratitude to Professor J. E. Jorpes, Dozent V. Mutt, and Dr. M. I. Grossman for the biological assays; to Mr. J. Alicino and his group for the microanalyses; to Mr. O. Kocy for the electrophoretic studies; and to Mr. F. Russo-Alesi for the quantitative amino acid analyses.

The Structure Determination of Antibiotic Compounds from *Hypericum uliginosum*. I[1]

William L. Parker and Francis Johnson

Contribution from the Dow Chemical Company, Eastern Research Laboratory, Wayland, Massachusetts 01778. *Received February 26, 1968*

Abstract: Structures for two antibiotic compounds, uliginosin A (I) and uliginosin B (II), have been deduced from a detailed study of the nmr, ir, uv, and mass spectra of these substances and of simple derivatives of them. The compounds contain phloroglucinol and filicinic acid residues and are closely related chemically to fern constituents such as aspidin and flavaspidic acid. The structures also resemble that of rottlerin which, like I and II, occurs in a higher plant.

Hypericum uliginosum HBK is a woody herb which is widely distributed in Mexico and Central America. In Mexico, where the plant is used for the treatment of diarrhea, it is called Tzotzil and also "rabbit plant." An investigation of the chemical constituents of the plant was undertaken as part of a general search for natural products with potentially useful biological activity. An extract of the plant showed antibacterial properties and purification of the extract by chromatography and countercurrent distribution led to the isolation of two pale yellow crystalline compounds, both of which showed good activity against gram-positive bacteria.[2] We have undertaken the elucidation of the structures of these antibiotics and have called the higher melting material (161.5°) uliginosin A (I) and the lower melting one (142.0°) uliginosin B (II). The spectra of these compounds indicate that they are closely related, but since the spectra of uliginosin B appeared more tractable, our initial efforts were directed to this substance.

Elemental analysis of uliginosin B was consistent with both $C_{31}H_{38}O_9$ and $C_{28}H_{34}O_8$ but an ebullioscopic molecular weight determination gave a value of 512 ± 25, more in agreement with the latter formula (MW = 498.60) rather than the former (MW = 554.66). Confirmation of this choice was obtained from the high-resolution mass spectrum of II (to be discussed in more detail later) which not only showed a parent ion peak at 498.2261 mass units but also revealed an impurity present to the extent of ~20% and having a molecular weight of 512.2406 ($C_{29}H_{36}O_8$). Efforts to remove this impurity by recrystallization were to no avail. Fortunately, the presence of this substance, once recognized, did not prove too much of a hindrance to the elucidation of the structure of II.

Catalytic reduction of uliginosin B gives rise to a dihydro derivative III ($C_{28}H_{36}O_8$), whereas bromination followed by pyridine dehydrobromination leads to a monobromo compound, $C_{28}H_{33}BrO_8$ (IV). Neither of these compounds could be freed completely from the corresponding derivatives of the impurity present in II. However, they were sufficiently pure for spectroscopic studies. Attempts to acetylate or methylate II did not lead to clean products, and these experiments were abandoned.

The infrared spectrum of II showed broad absorption in the 3-μ region and this, coupled with intense peaks from 1600 to 1650 cm^{-1}, suggested the presence of an enolic 1,3-diketo system or a 2-hydroxyaryl ketone.[3,4] Although II did not give a distinct ferric chloride test (brown precipitate) the formation of a copper chelate confirmed the presence of such a group. It is noteworthy that recrystallization of the chelate followed by regeneration of II by treatment with sodium sulfide did not lead to an improvement in the purity of II.

The nmr spectrum (Figure 1) of uliginosin B proved most informative since it contains several very interesting features that reveal a number of important structural details. First and foremost, the multiplet at 4 ppm is well resolved at 100 Mc and is easily recognized as two overlapping septets, one centered at 4.19 ppm (J = 6.6 cps) and the other at 3.85 ppm (J = 6.6 cps). Although the high-field septet appears the more intense, integration indicates that to all intents and purposes, each is caused by one proton. These multiplets are coupled with superimposed doublets at 1.17 ppm (J = 6.7 cps) which integrate for 12 protons. Taken in conjunction, these features indicate two isopropyl groups. The septets occur in a region that is typical for an isopropoxy group. However, since the latter function rarely occurs in natural products, it seemed more reasonable to regard these peaks as being due to isopropyl ketone moieties even though the >C*H*– of this group generally absorbs at higher fields.[5a,6a]

(1) Paper I in a series of two papers.

(2) The isolation work was carried out by Drs. R. M. Brooker and H. L. Taylor of the Dow Human Health and Development Center, Zionsville, Ind., who will report their procedure and their biological test results in a separate publication.

(3) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p 43.

(4) S. Forsén, F. Merenyi, and M. Nilsson, *Acta Chem. Scand.*, **18**, 1208 (1964).

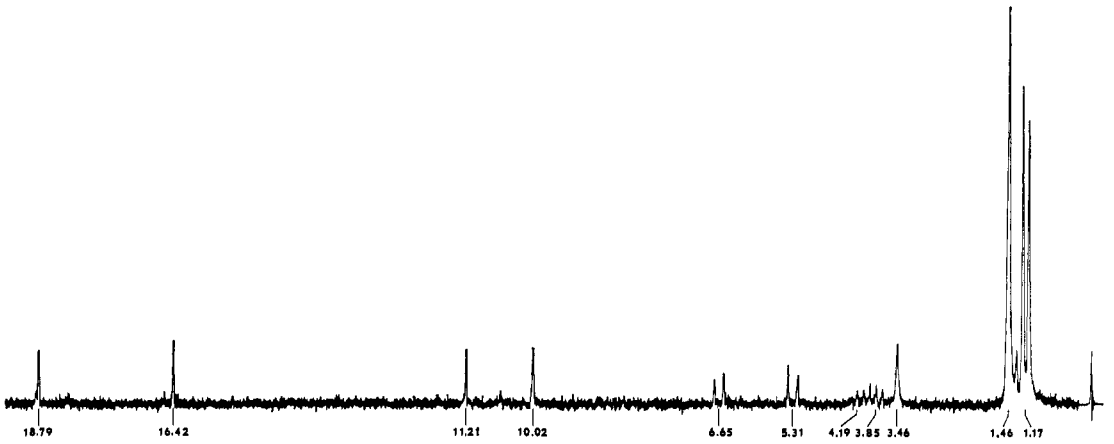
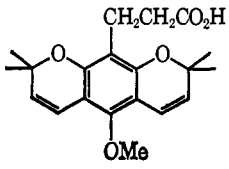


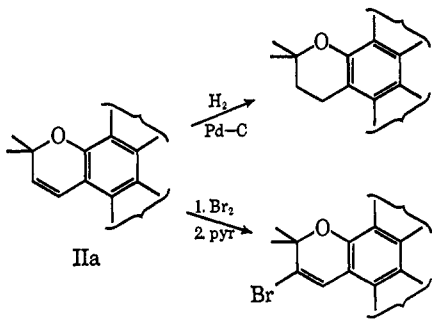
Figure 1. Nmr spectrum of uliginosin B.

The doublets at 6.65 and 5.31 ppm are coupled (J = 9.9 cps), and each have intensities corresponding to one proton. By contrast, these signals are missing from the spectrum of dihydrouliginosin B (III), where they are replaced by two coupled triplets (J = 6.8 cps) at 2.63 and 1.76 ppm. The location and coupling of the doublets exhibited by II are so close to those found for the olefinic protons of eriostoic acid (V) [6.53 and 5.50 ppm (J = 9.9 cps)][5b] as to suggest very strongly that uliginosin B contains a dimethylchromene system. The aromatic ring in II must be fully substituted since



V

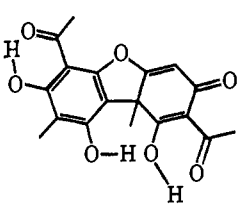
no aromatic protons are evident in the spectrum. Catalytic reduction must involve the double bond in the pyran ring (IIa) as does bromination because the nmr spectrum of bromouliginosin B (IV) shows a singlet at 7.04 ppm typical of a β-bromostyryl group, the coupled doublets characteristic of II being absent.



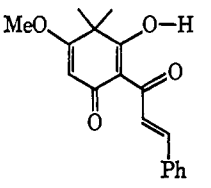
IIa

The singlet at 1.46 ppm in the nmr spectrum of II can be accounted for in part by the *gem*-dimethyl group of the chromene system. However, this peak has a shoulder on the low-field side that sharpens into a separate peak on warming or cooling. (This behavior will be discussed later.) This temperature dependence can be seen more clearly in dihydrouliginosin B (III) because the peak in question, now at 1.50 ppm, is no longer complicated by the methyl group absorption of the heterocyclic ring. The latter appears at 1.38 ppm. Integration of both spectra from ~1.6 to 1.0 ppm reveals the presence of 24 protons, 18 of which have already been accounted for. The temperature-dependent peak thus appears to be due to two other closely related methyl groups.

Perhaps the most interesting aspect of the nmr spectrum of II is the presence of four singlets at 10.02, 11.21, 16.42, and 18.79 ppm, each accounting for one proton. All must be due to hydroxylic protons because they are absent from the spectrum of a sample of II that has been shaken briefly with deuterium oxide. The peak at 18.79 ppm is at a remarkably low position but as such proved very informative since β-triketones constitute one of the few systems known which absorb in this region. For example, both usnic acid[7] (VI) and ceroptene[8] (VII) exhibit a peak at ~19 ppm, and in a spectral study[4] of β-triketones, it has been found that an enolic proton in this environment gives a signal in the 17–19-ppm region when two of the ketone groups are in a six- or a seven-membered ring or when the

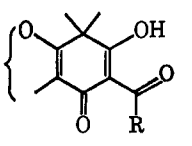


VI



VII

system is acyclic. This correlation, together with the fact that the *gem*-dimethyl group of VII absorbs at 1.5 ppm in the nmr spectrum,[8] strongly suggests that II contains a 3-acylfilicinic acid residue (VIII).



VIII

(5) NMR Spectra Catalog, Varian Associates, Palo Alto, Calif., 1963: (a) spectra 543 and 559; (b) spectrum 344.

(6) The Sadtler Standard Spectra, Sadtler Research Laboratories, Philadelphia, Pa., 1967: (a) spectra 1421, 1865, and 1885; (b) spectra 361, 511, 985, and 1556.

(7) S. Forsén, M. Nilsson, and C. A. Wachtmeister, *Acta Chem. Scand.*, **16**, 583 (1962).

(8) S. Forsén and M. Nilsson, *ibid.*, **13**, 1383 (1959).

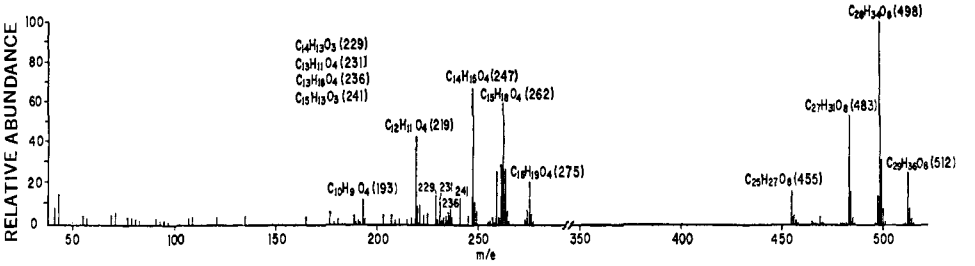


Figure 2. Mass spectrum of uliginosin B.

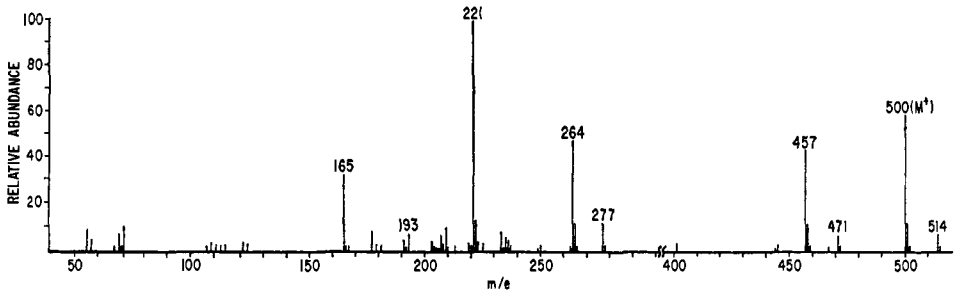
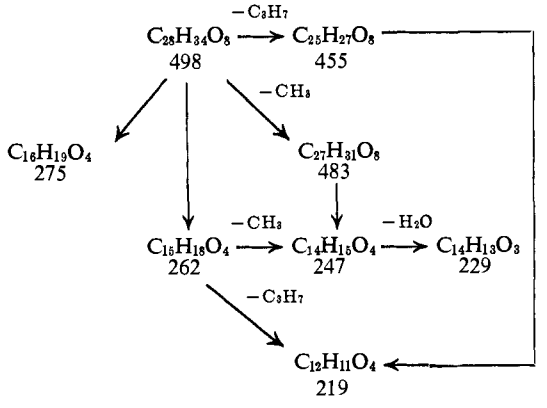


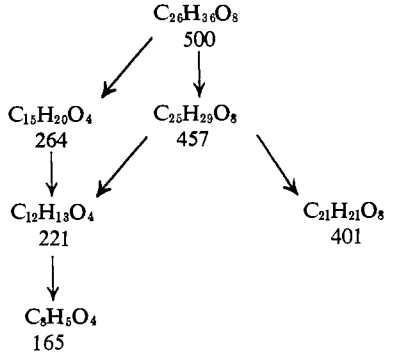
Figure 3. Mass spectrum of dihydrouliginosin B.

Thus far, of the 34 protons in II, 32 have been tentatively assigned to various functions. The remaining two appear as a slightly broadened singlet at 3.46 ppm.

Further information was obtained from the analysis of the mass spectra of II and III (Figures 2 and 3, respectively). Metastable transitions, some of which were determined from pure metastable spectra,[9] indicated the following fragmentation routes for II.

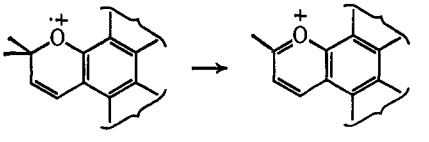


Likewise, the fragmentation routes shown below were determined for III.



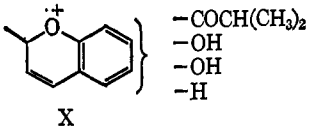
A major fission of both of these compounds under electron impact involves cleavage into two roughly equal pieces. Most of the major low mass ($m/e < 280$) peaks in the spectrum of uliginosin B have corresponding peaks two mass units higher in the spectrum of its dihydro derivative, indicating that these peaks are derived from the 2,2-dimethylchromene system. However, a strong peak in the spectra of both compounds occurs at m/e 193, and undoubtedly is associated with the other half of the molecule.

A distinct difference between II and III is the very facile loss of methyl (M − 15) that the former shows. This cleavage can be accounted for by the aromatic stabilization[10] afforded the product IX, an effect that cannot operate for III. The decomposition of the



IX

fragment at m/e 262 to that at m/e 247 in the spectrum of II is of the same type since its counterpart scarcely exists in the spectrum of III. We interpret this and other features of the spectra to indicate that the $C_{15}H_{18}O_4$ fragment (X) at 262 mass units in the spectrum of II consists of the 2,2-dimethylchromene system substituted with an isobutyryl function and two hydroxyl groups. In addition it contains also a hydrogen atom transferred during the fragmentation.



X

We observed (initially with some concern) that neither II nor III lost isobutyryl groups to give

(9) M. Barbar and R. M. Elliott, Abstracts of Twelfth Annual Conference on Mass Spectrometry and Allied Topics, Montreal, Canada, June 1964, p 155.

(10) C. S. Barnes, J. L. Occolowitz, N. L. Dutta, P. M. Nair, P. S. Phadke, and K. Venkataraman, *Tetrahedron Letters*, 281 (1963).

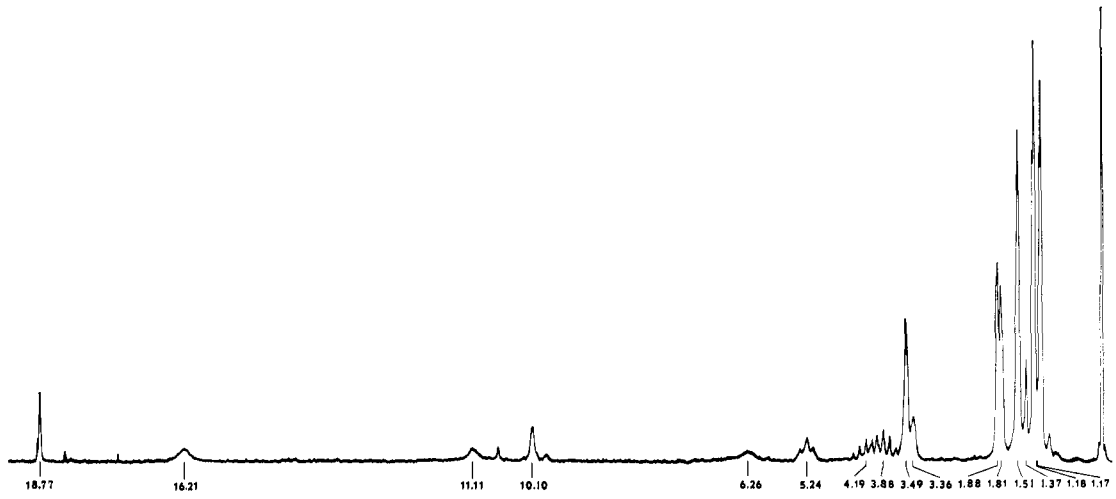
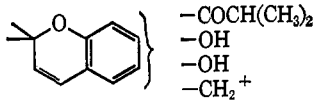


Figure 4. Nmr spectrum of uliginosin A.

peaks at 427 or 429 mass units, respectively. However, this mass spectral behavior is similar to that of 2′,4′,6′-trihydroxyacetophenone and is to be contrasted with that of 2′-hydroxyacetophenone and 2′,4′-dihydroxyacetophenone, both of which give major peaks for the loss of acetyl.

The fragment (XI) at *m/e* 275 ($C_{16}H_{19}O_4$) must also contain the 2,2-dimethylchromene system but has a methylene group in place of the nonaboriginal hydrogen atom of X. Undoubtedly, this methylene group is

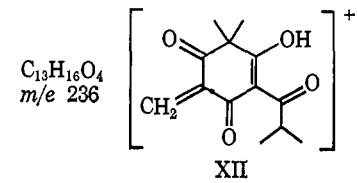


XI

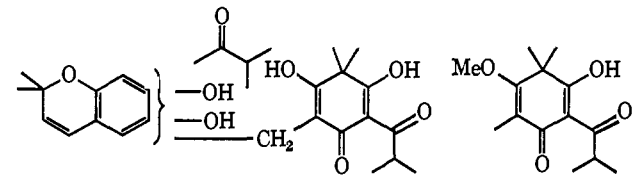
responsible for the signal at 3.46 ppm in the nmr spectrum of II.

The pure metastable spectrum of the 193 mass unit ($C_{10}H_9O_4$) fragment from uliginosin B indicates that it comes from a relatively minor ion with a mass of 236. High-resolution mass measurement indicates that this latter ion is $C_{13}H_{16}O_4$. We feel that this ion very likely constitutes the remainder of uliginosin B and is attached to the chromene moiety by the methylene group of structure XI. The remaining functional groups that have been detected in the nmr spectrum must be present in the $C_{13}H_{16}O_4$ fragment. These are an isobutyryl and two hydroxyl groups and another oxygen atom, which must in some way be incorporated to give an enolic β-triketone. The data presented up to this point give no guidance for putting these groups and the remaining atoms together in the correct arrangement. However, as pointed out before, arrangement of these groups to form a 3-acylfilicinic acid would be eminently reasonable. This hypothesis leads to XII as the structure for the mass 236 fragment and to XIII as an embryonic structure of uliginosin B. In the mass spectra of II and III, the peaks at 193 mass units are thus related to those at 236 mass units by the loss of isopropyl groups. The structure of the filicinic acid moiety is similar to that of tasmanone, XIV, an essential oil from *Eucalyptus risdoni* and *Eucalyptus linearis*.[11]

Permutation of the four groups attached to the aromatic ring can give 12 possible isomers for consideration. However, the infrared spectrum of uliginosin B has no absorption peak above 3160 cm^{-1} and shows no change upon dilution, indicating that all the hydroxyl protons are intramolecularly chelated. All of the 12 isomers can be drawn with the four hydroxyl protons near neighboring oxygen. However, eight of the structures are considered unlikely in that they involve five-membered chelate rings that would be expected to give an absorption peak at about 3575–3535 cm^{-1} in the infrared spectrum.[12] Five of these eight structures are rendered even more unlikely in that they contain a carbonyl group which is not hydrogen bonded and which would therefore be expected to give infrared absorption for a normal dienone[3] at about 1665 cm^{-1}.



XII



XIII XIV

At this point, discussion of the higher melting antibiotic, uliginosin A (I), is productive. The nmr spectrum of uliginosin A (Figure 4) differs markedly from that of uliginosin B (Figure 1) in that the former contains two narrowly separated peaks at 1.8 ppm (six protons) and a triplet (one proton) at 5.2 ppm while the absorption near 3.5 ppm now integrates for four protons rather than two. These features indicate an isopentenyl group attached to an aromatic ring. The spectrum also lacks the AB quartet characteristic of uliginosin B and has a rather broad peak due to one hydroxylic proton at 6.26 ppm. The singlet at 1.51 ppm is noticeably weaker with respect to the doublet at 1.17 ppm, in comparison to the corresponding peaks in the spectrum of uliginosin B. The

(11) A. J. Birch and P. Elliott, *Australian J. Chem.*, 9, 95 (1956).

(12) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, p 103.

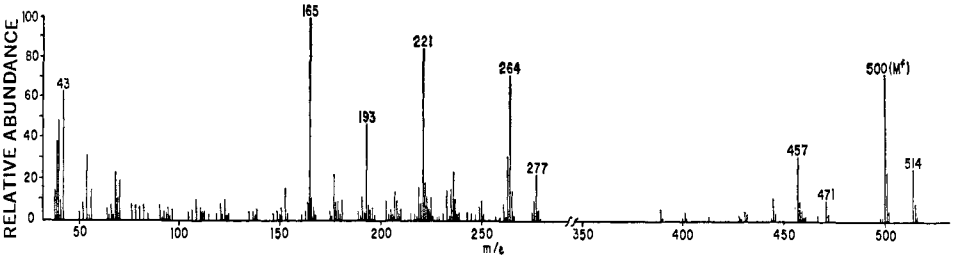
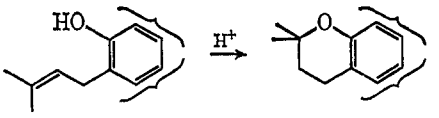


Figure 5. Mass spectrum of uliginosin A.

mass spectrum of uliginosin A (Figure 5) is remarkably similar to that of dihydrouliginosin B (Figure 3) and shows a parent ion peak at *m/e* 500 (peaks at higher mass numbers being due to impurities which are probably higher homologs of I). From these observations, it seemed likely that uliginosin A has the same structure as dihydrouliginosin B, except that an isopentenyl side chain and an hydroxyl group take the place of the oxygen heterocycle.

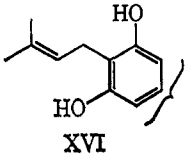
On the basis of this deduction, uliginosin A was treated with *p*-toluenesulfonic acid in boiling benzene in the hope that the proposed *o*-hydroxyisopentenylbenzene system (Ia) would give the dihydropyran ring of dihydrouliginosin B (III). This reaction not only



Ia

afforded III but gave in almost equal yield an isomeric compound which we have termed isodihydrouliginosin B (XV). This substance has an nmr spectrum that is much like that of dihydrouliginosin B, but several chemical shifts are significantly different. The largest difference involves the hydroxylic protons which absorb at 16.51 and 10.10 ppm in dihydrouliginosin B but which are now found at 14.05 and 9.30 ppm in the spectrum of XV. The mass spectrum of XV also looks much like the spectra of dihydrouliginosin B and uliginosin A.

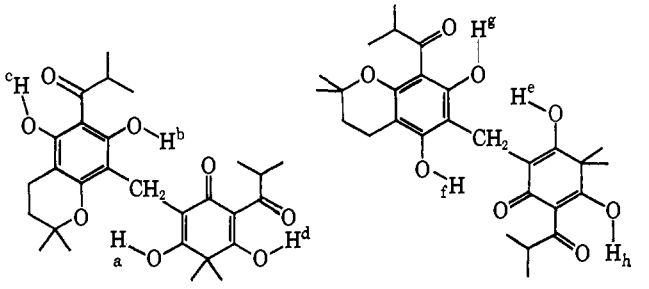
The formation of XV is best rationalized in terms of the partial structure XVI for uliginosin A. This system can now cyclize in two directions, one to give dihydrouliginosin B, and the other to give the isomer, isodihydrouliginosin B. This partial structure is par-

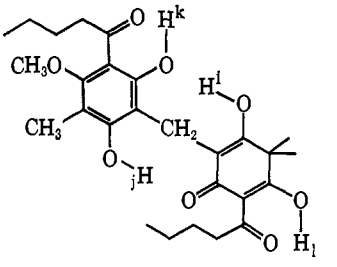


XVI

ticularly helpful in that it provides solid evidence for eliminating two of the four possible structures for uliginosin B. The infrared spectrum of isodihydrouliginosin B at high dilution indicates that all of the hydroxylic protons are involved in intramolecular hydrogen bonds.

Dihydrouliginosin B and isodihydrouliginosin B each must then be assigned one of the structures XVII and XVIII. The decision as to which compound corresponds to which structure can be arrived at by making detailed assignments of the chelated protons in the nmr spectra of these compounds. Comparative data for this purpose were obtained from the nmr spectrum of aspidin[13] (XIX), an antibiotic fern constituent of well-established structure.[14] The obser-





XIX

vations that the structure of aspidin is closely analogous to structure XVIII and that the nmr spectra of aspidin and dihydrouliginosin B are quite similar led to the conclusion that dihydrouliginosin B has structure XVIII and that isodihydrouliginosin B has structure XVII. The nmr assignments are shown in Table I.

Table I. Chemical Shifts of Hydroxylic Protons Measured in CCl_4

Proton	Isodihydrouliginosin B (XXII)	Proton	Dihydrouliginosin B (XXIII)	Proton	Aspidin (XXIV)
a	9.30	e	10.17	i	9.96
b	11.02	f	11.00	j	11.10
c	14.05	g	16.51	k	15.81
d	18.80	h	18.78	l	18.65

The very low-field absorption due to the β-tricarbonyl system (protons d, h, and l) has already been discussed. Protons b, f, and j occupy nearly identical environments in the three structures and hence have nearly the same chemical shifts. Proton a is involved in the weakest

(13) Samples of this material were kindly supplied to us by A. Penttilä, Medica Ltd., Helsinki, Finland, and by the Smith Kline and French Research Institute, Welwyn Garden City, England.

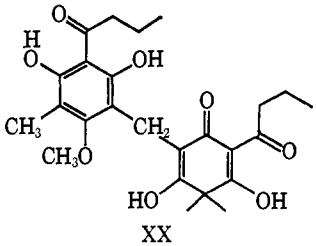
(14) W. Ried and R. Mitteldorf, *Chem. Ber.*, 89, 2595 (1956).

hydrogen bond and is therefore found at the highest field.[15] The relative strength of this hydrogen bond is also reflected in the hydroxyl region of the infrared spectrum of isodihydrouliginosin B. The maximum for this compound in carbon tetrachloride is at 3198 cm^{-1} while the maximum for dihydrouliginosin B is at 3142 cm^{-1}. A particularly interesting relationship is found between protons g and e and between protons k and i in that the hydrogen-bonded atoms form chains similar to those proposed for polymeric association in alcohols.[16] This arrangement could be used to rationalize the large downfield shift of protons g and k relative to c. A number of simple *o*-hydroxyphenones show absorption for the chelated hydrogen at 12 to 12.5 ppm[6b] but it has been demonstrated that further oxygenation of the aromatic ring causes a shift to lower field. For example, in 2′-hydroxy-4′,6′-dimethoxyacetophenone, the chelated proton is found at 14.03 ppm,[7] in good agreement with the absorption assigned to proton c in isodihydrouliginosin A.

The comparisons shown in Table II of the ultraviolet spectra of aspidin[17] and dihydrouliginosin B and of paraaspidin[17] (XX) and isodihydrouliginosin B provide reasonable correlations.

Table II. Ultraviolet Spectra in Cyclohexane

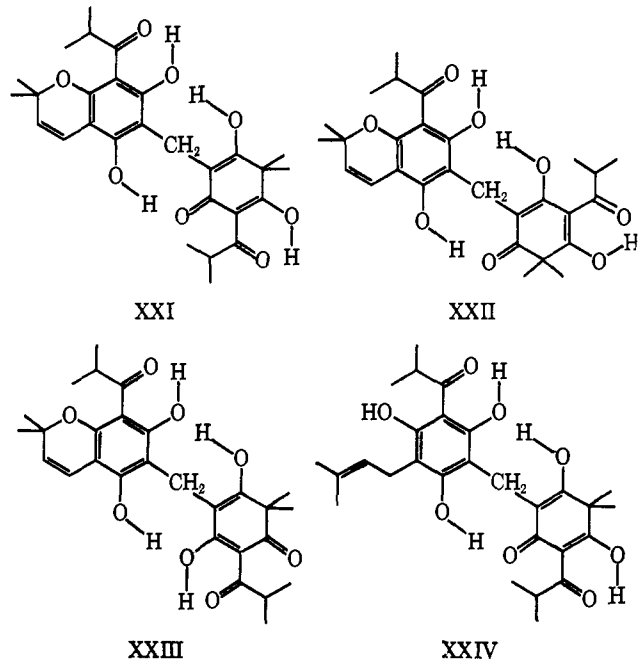
	λ_{max}, mμ	log ε
Aspidin	230, 292	4.43, 4.30
Dihydrouliginosin B	232, 300	4.40, 4.35
Paraaspidin	228, 271	4.43, 4.33
Isodihydrouliginosin B	231, 283	4.39, 4.29



XX

The conclusions most consistent with the data presented are that the structure of uliginosin B is XXI or one of the tautomers, XXII and XXIII, and that the structure of uliginosin A is XXIV (or a tautomer).

As alluded to several times above, the nmr spectra of the uliginosin compounds have certain anomalies that can be explained on the basis of tautomerization which is expected for the acylfilicinic acid system.[7] This tautomerization was considered in detail during examinations of the peak at 1.50 ppm in the nmr spectrum of dihydrouliginosin B (XVIII) at various temperatures. This peak, which is a singlet at room temperature and above but a doublet at low temperatures, was assigned to the *gem*-dimethyl group of the filicinic acid moiety. Careful integration of these absorptions indicates that they are caused by *less* than six protons and that the peak at 1.38 ppm (largely due to the *gem*-dimethyl group of the oxygen heterocycle) is caused by more than six protons. The nmr spectrum of uliginosin B (Figure 1) has a minor peak at 1.33 ppm which has been ignored in the previous discussion. This peak is no doubt due to the *gem*-dimethyl group of the filicinic acid moiety in a minor tautomer. The peak does broaden upon cooling, but this region of the spectrum is not clear enough to observe the absorption at low temperatures where it should be a doublet. The difference between the chemical shifts of the peak at 1.50 ppm in dihydrouliginosin B and the peak at 1.33 ppm in uliginosin B is in line with the differences found for tautomers of ceroptene and related compounds.[7] If it is assumed that the corresponding peak for the minor tautomer of dihydrouliginosin B is under the peak (1.38 ppm) for the *gem*-dimethyl group of the oxygen heterocycle, integration of this region indicates that the major tautomer is present to the extent of approximately 74% at 60° and 81% at −45°. However, an element of uncertainty is added by the presence of the impurity (*ca.* 20%) detected in the mass spectrum.



XXI XXII XXIII XXIV

Tautomer XXIII of uliginosin B is unlikely as a major component since the carbonyl group which is not hydrogen bonded in this structure should give a peak in the infrared spectrum at a shorter wavelength (*ca.* 1665 cm^{-1}) than the observed absorption. However, a minor contribution from this tautomer may be indicated[4] by the very weak shoulder at about 1670 cm^{-1}.

The temperature-dependent absorption at 1.50 ppm in the nmr spectrum of dihydrouliginosin B (XVIII) (Figure 6) indicates protons in two different environments which become equivalent by time averaging upon warming. The two methyl groups of the filicinic acid moiety exchange environments when the two ring systems are rotated by 180° about their bonds to the connecting methylene group. This involves the rupture of two hydrogen bonds in the transition state. The methyl groups on the oxygen heterocycle should also be nonequivalent at low temperatures, but the environments are so similar that only one peak is observed.

(15) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, Chapter 15.

(16) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 473.

(17) A. Penttilä and J. Sundman, *Acta Chem. Scand.*, **16**, 1251 (1962).

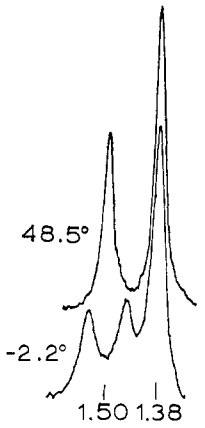
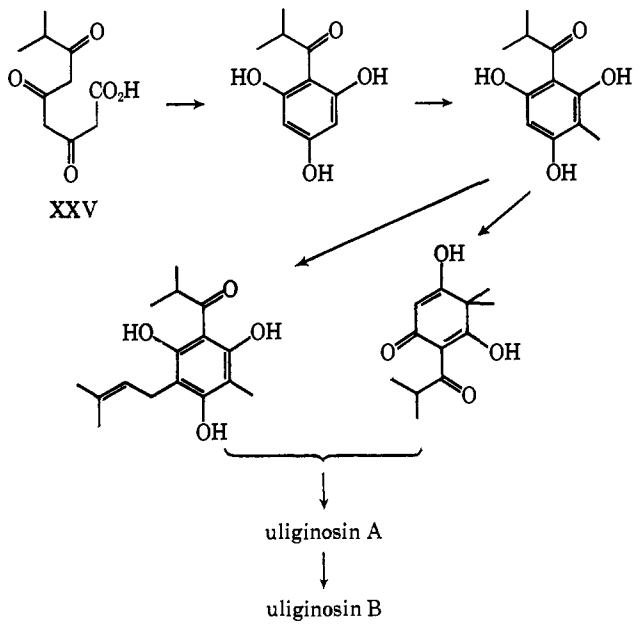


Figure 6. Variable-temperature nmr spectrum of dihydrouliginosin B.

Finally it should be noted that biogenetically, the synthesis of the uliginosins appears to involve a polyketide, mevalonate, and methionine. The two carbocyclic rings may be formed by cyclization of a linear β-triketo acid intermediate XXV[18] whereas the isopentenyl side chain of uliginosin A and the corresponding five carbon atoms in the chromene system of uliginosin B are probably derived from mevalonate.[19] The methylene bridge and the methyl groups of the filicinic acid moiety undoubtedly are derived from methionine[20] prior to condensation of filicinic acid and



phloroglucinol intermediates to give uliginosin A. This type of condensation has been elegantly demonstrated in the recent work of Penttilä and Fales on the *in vitro* enzymic synthesis of methylenebisphloroglucinol derivatives related to aspidin.[21]

(18) J. L. Douglas and T. Money, *Tetrahedron*, 23, 3543 (1967), and references contained therein.

(19) J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Benjamin, Inc., New York, N. Y., 1964, p 23.

(20) A. Penttilä, G. J. Kapadia, and H. M. Fales, *J. Am. Chem. Soc.*, 87, 4402 (1965).

(21) A. Penttilä and H. M. Fales, *ibid.*, 88, 2327 (1966).

Experimental Section

Melting points were determined in glass capillaries and are corrected. Nuclear magnetic resonance spectra were taken at 36° in carbon tetrachloride on Varian A56-60 and HR100 spectrometers. Chemical shifts are reported in parts per million downfield from a tetramethylsilane internal standard. Infrared spectra were taken in carbon tetrachloride solution on a Perkin-Elmer Model 337 spectrophotometer. Ultraviolet spectra were determined in cyclohexane using Perkin-Elmer Model 202 and Cary Model 14PM spectrophotometers. Mass spectra were taken on CEC 21-110B and MS-12 spectrometers. Elemental analyses were determined by Galbraith Laboratories, Inc. Samples of uliginosin A and B were obtained from The Human Health Research and Development Center, The Dow Chemical Company.

Uliginosin A. Uliginosin A which had been recrystallized several times from acetonitrile–chloroform solution (4:1) melted at 160.5–161.5°.

Anal. Calcd for $C_{28}H_{36}O_8$: C, 67.18; H, 7.25. Found: C, 67.20; H, 7.18.

Nmr spectrum: 1.17, 1.18 (overlapping doublets, J = 6.5 cps, 12 H); 1.37 (weak), 1.51 (singlets, 6 H); 1.81, 1.88 (singlets, 6 H); 3.36 (rather broad), 3.49 (singlet), 3.88, 4.19 (septuplets, 6 H for these four shifts); 5.24 (triplet, J = 5.5 cps, 1 H); 6.26 (broad singlet, 1 H); 10.10 (singlet, 1 H); 16.21 (broad singlet, 1 H); 18.77 (singlet, 1 H); 11.11 (broad singlet, 1 H).

ν_{max}: 3580 (w), 3323 (m, broad), 3128 (m, broad), 2958 (s), 2920 (m), 2858 (m), 2653 (m, broad), 1639 (vs), 1610 (vs), 1576 (s), 1523 (m), 1473 (s), 1431 (s), 1380 (s), 1353 (s), 1293 (s), 1266 (s), 1193 (s), 1096 (m), and 915 (m) cm^{-1}.

λ_{max} (ϵ): 229 (31,500) and 293 mμ (25,000).

Uliginosin B. Recrystallization from nitromethane gave yellow platelets, mp 139.5–142.0°.

Anal. Calcd for $C_{28}H_{34}O_8$: C, 67.45; H, 6.87; O, 25.67. Found: C, 67.20, 67.40; H, 6.98, 6.84; O, 25.71, 25.82.

Nmr spectrum: 1.17 (doublet, J = 6.7 cps, 12 H), 1.33 (singlet, very weak), 1.46 (singlet, 12 H), 3.46 (singlet, 2 H), 3.85 (septet, J = 6.6 cps, 1 H), 4.19 (septet, J = 6.6 cps, 1 H), 5.31 (doublet, J = 9.8 cps, 1 H), 6.65 (doublet, J = 9.9 cps, 1 H), 10.02 (singlet, 1 H), 11.21 (singlet, 1 H), 16.42 (singlet, 1 H), 18.79 (singlet, 1 H). The last four peaks disappear when the sample is shaken with D_2O.

λ_{max} (ϵ): 230 (34,000) and 270 mμ (37,000).

ν_{max}: 3153 (broad), 2971 (s), 2931 (m), 2869 (m), 1644 (vs), 1604 (vs), 1531 (m), 1476 (s), 1432 (s), 1385 (s), 1364 (s), 1279 (s), 1191 (s), 1128 (s), 914 (m) cm^{-1}.

High-resolution mass measurements [empirical formula, exptl, (calcd)]: $C_{29}H_{36}O_8$, 512.2406, 512.2407, (512.2410); $C_{28}H_{34}O_8$, 498.2261, (498.2254); $C_{27}H_{31}O_8$, 483.2018, (483.2018); $C_{25}H_{27}O_8$, 455.1712, (455.1705); $C_{16}H_{19}O_4$, 275.1293, (275.1283); $C_{15}H_{18}O_4$, 262.1214, 262.1219, (262.1205); $C_{14}H_{13}O_4$, 247.0962, (247.0970); $C_{15}H_{13}O_3$, 241.0875, (241.0865); $C_{13}H_{16}O_4$, 236.1054, (236.1048); $C_{13}H_{11}O_4$, 231.0660, (231.0657); $C_{14}H_{13}O_3$, 229.0867, (229.0865); $C_{12}H_{11}O_4$, 219.0662, (219.0657); $C_{10}H_{19}O_4$, 193.0504, (193.0501).

Dihydrouliginosin B. Uliginosin B (93 mg) in 10 ml of ethyl acetate was hydrogenated over 26 mg of 10% palladium on charcoal. The theoretical amount of hydrogen was taken up in 4 min, and no further uptake was evident. The mixture was filtered and the solvent removed *in vacuo*. The residue (92 mg) was recrystallized several times from acetonitrile–chloroform (9:1) to give material melting at 138–141°.

Anal. Calcd for $C_{28}H_{36}O_8$: C, 67.18; H, 7.25. Found: C, 67.24; H, 7.13.

Nmr spectrum: 1.17, 1.19 (pair of doublets, J = 6.7 cps, 12 H); 1.38, 1.50 (singlets, 12 H); 1.76 (triplet, J = 6.8 cps, 2 H); 2.63 (triplet, J = 6.9, 2 H); 3.48 (singlet, 2 H); 3.82 (septet, J = 6.7 cps, 1 H); 4.21 (septet, J = 6.6 cps, 1 H); 10.17 (singlet, 1 H); 11.00 (singlet, 1 H); 16.51 (singlet, 1 H); 18.78 (singlet, 1 H).

λ_{max} (ϵ): 232.0 (25,300) and 299.5 mμ (22,300).

ν_{max}: 3142 (s, broad), 2702 (w, broad), 2972 (s), 2932 (s), 2867 (m), 1642 (vs), 1612 (vs), 1578 (s), 1477 (m), 1427 (s), 1382 (s), 1372 (s), 1352 (m), 1275 (s), 1192 (s), 1156 (s), 1138 (m), 1116 (s), 1040 (m), and 914 (w) cm^{-1}.

Isodihydrouliginosin B. Uliginosin A (35 mg) was dissolved in 1 ml of benzene, and the solution was saturated with HCl at room temperature. Thin layer chromatography after 15 hr showed that only partial reaction had taken place with two products being formed. The solvent was removed *in vacuo*, and the product in 1 ml of benzene was refluxed with 4.7 mg of *p*-toluenesulfonic acid for 30 min to complete the reaction. The solvent was removed *in vacuo*, and the residue was dissolved as much as possible in a little

hexane. This solution was chromatographed on 5 g of silica gel eluting with benzene–hexane–formic acid (50:50:1). The first band (11 mg) was dihydrouliginosin B, identified by chromatographic and spectroscopic comparison with authentic material. The second band (10.5 mg) was obtained as an amorphous powder when a methanol solution was taken to dryness *in vacuo*. Recrystallization from hexane–ethyl acetate gave material which melted at 156.0–158.5°.

Anal. Calcd for $C_{28}H_{36}O_8$: C, 67.18; H, 7.25. Found: C, 67.12; H, 7.23.

Nmr spectrum: 1.20, 1.22 (overlapping doublets, J = 6.7 cps); 1.50 (singlet); 1.54 (singlet); 1.86 (triplet, J = 6.2 cps); 2.62 (triplet, J = 6.5 cps); 3.48 (singlet, 2 H); 4.10 (septuplet, J = 6.9 cps, 2 H); 9.30 (singlet); 11.02 (singlet); 14.05 (singlet); 18.80 (singlet).

λ_{max} (ϵ) (cyclohexane): 231 (24,600) and 283 mμ (19,500).

ν_{max} (carbon tetrachloride): 3198 (broad), 2723 (w, broad), 2660 (w, broad), 2973 (s), 2930 (s), 2868 (w), 1640 (s), 1603 (vs), 1483 (m), 1422 (s), 1379 (m), 1348 (m), 1282 (m), 1259 (m), 1237 (m), 1190 (m), 1155 (m), 1137 (m), and 1113 (m) cm^{-1}.

Bromouliginosin B. Uliginosin B (48.7 mg, 0.0978 mmol) was dissolved in 1 ml of carbon tetrachloride. The solution was cooled to 0° and 0.96 ml of a 0.102 M solution of bromine in carbon tetrachloride (0.098 mmol of bromine) was added. The solvent was immediately removed *in vacuo* to give a pale yellow, noncrystalline residue. A solution of this material in 2 ml of pyridine was heated at 100° for 1 hr. The pyridine was neutralized with excess 10% sulfuric acid, and the product was extracted from this mixture with dichloromethane. Removal of the solvent gave 59.3 mg of a dark orange gum which was chromatographed on silica gel. Benzene rapidly eluted 38.7 mg of a pale yellow material. After several recrystallizations from acetonitrile, this material melted at 155.0–158.0° and had a density (by density gradient) of 1.377 g/cm^3.

Anal. Calcd for $C_{28}H_{33}BrO_8$: C, 58.23; H, 5.76; Br, 13.84. Found: C, 58.57; H, 5.91; Br, 13.84.

λ_{max} (ϵ): 234 (27,000) and 290 mμ (34,000).

ν_{max}: 3170 (broad), 2979 (s), 2933 (m), 2868 (w), 2637 (w), 1642 (vs), 1605 (vs), 1479 (m), 1433 (w), 1387 (s), 1367 (m), 1275 (s), 1193 (s), 1126 (s), 1039 (w), and 928 (w) cm^{-1}.

Nmr spectrum: 1.20 (doublet, J = 6.5 cps, 12 H), 1.35 (weak), 1.51 (slightly broad singlet, 6 H), 1.62 (sharp singlet, 6 H), 3.50 (broad singlet, 2 H), 4.0 (complex multiplet, 2 H), 7.04 (singlet, 1 H), 9.89 (singlet, 1 H), 11.47 (singlet, 1 H), 16.39 (singlet, 1 H), 18.90 (singlet, 1 H).

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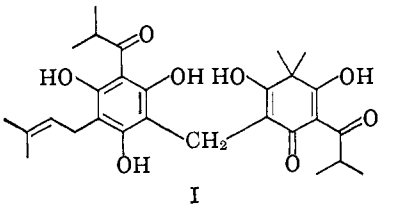
The Structure Determination of Antibiotic Compounds from *Hypericum uliginosum*. II.[1] The Molecular and Crystal Structure of Bromouliginosin B

William L. Parker, J. J. Flynn, and F. P. Boer

Contribution from the Dow Chemical Company, Eastern Research Laboratory, Wayland, Massachusetts 01778. *Received February 26, 1968*

Abstract: The crystal and molecular structure of bromouliginosin B, $C_{28}H_{33}BrO_8$, a derivative of an antibiotic from *Hypericum uliginosum*, has been determined by a single-crystal, three-dimensional X-ray diffraction study. The compound crystallizes in the centrosymmetric space group $P\bar{1}$, and there are two molecules in each triclinic unit cell of dimensions a = 10.44, b = 10.79, c = 13.37 Å; α = 103° 37′, β = 99° 11′, and γ = 102° 44′. The intensity data were obtained on a Picker automatic diffractometer and the structure was solved by the heavy atom method. Least-squares refinement including anisotropic thermal motion converged to a discrepancy index R = 10.6%. The structure consists of filicinic acid and phloroglucinol moieties linked by a methylene bridge and held in a rigid conformation by two hydrogen bonds. Two other hydrogen bonds with unusually short oxygen–oxygen distances occur in the molecule, and these correlate with very low nmr chemical shifts for the chelated protons.

Uliginosins A (I) and B (IIa) are antibiotics isolated from *Hypericum uliginosum*, a woody herb found in Mexico and Central America. The detailed structure is reported here as determined for a suitable heavy atom derivative by single-crystal X-ray diffraction methods. This investigation was undertaken concurrently with the chemical studies[1] for several reasons. First, we wished to support the logic leading to postulated structures for uliginosins A and B based on the available spectroscopic and chemical evidence. An X-ray study appeared to be less time consuming and more rewarding than confirmation of the structure by traditional degradative methods. Synthesis also appeared to be a less attractive route to proof of structure because of published work on the synthesis of fern constituents that are closely related chemically to these compounds.[2,3] A second reason for undertaking the



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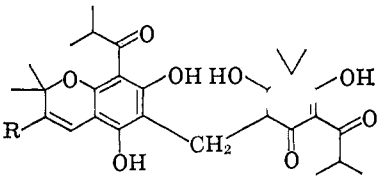

IIa, R = H
b, R = Br

(1) Paper I: W. L. Parker and F. Johnson, *J. Am. Chem. Soc.*, **90**, 4716 (1968).