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The Delineation of Griseofulvin and Related Systems by Nuclear Magnetic Resonance Spectroscopy

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Griseofulvin and various derived structural types related to the grisane nucleus have been defined by nuclear magnetic resonance spectroscopy. Support is also provided for the configurations assigned on chemical grounds to griseofulvin and epigriseofulvin.

In the course of our synthetic investigations of griseofulvin and various derivatives possessing structural modifications of the grisane nucleus,[1] nuclear magnetic resonance (n.m.r.) was found to be of great assistance in defining isomeric and stereochemical differences and thereby the particular reaction course pursued. The application of n.m.r. spectroscopy to the griseofulvin molecule is exemplary in the degree to which this structure and its relatives can be accurately and unequivocally defined.[2] In large measure, the interpretative problems were simplified by the relatively few and generally discrete proton types which characterize the various members of the series. Tables I and II list the n.m.r. data for the griseofulvins together with a number of related benzophenones, esters and simple aromatic precursors.

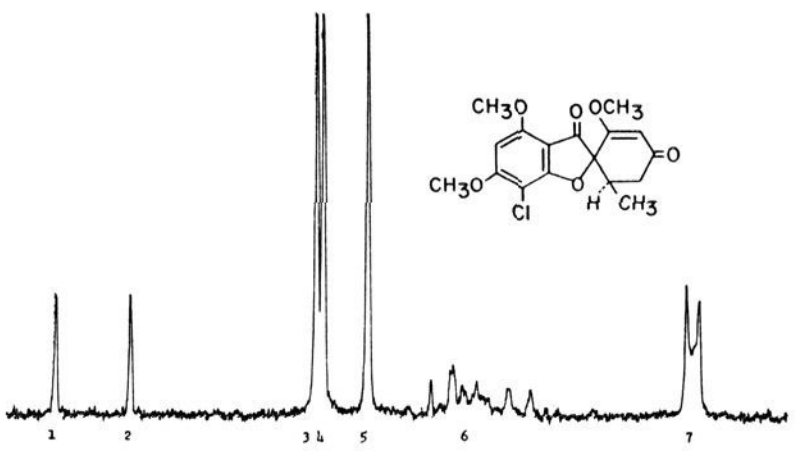


Fig. 1.—N.m.r. spectrum of griseofulvin (5% in $CDCl_3$).

Band	Value[a]	Functionality
1	3.85	Aromatic H
2	4.49	Vinyl H
3	5.96	Aromatic OCH_3
4	6.01	
5	6.37	Vinyl OCH_3
6	7.3(m)	CH_2–CH
7	9.02 (J = 6 c.p.s.)	C–CH_3

[a] Tetramethylsilane = 10.0.

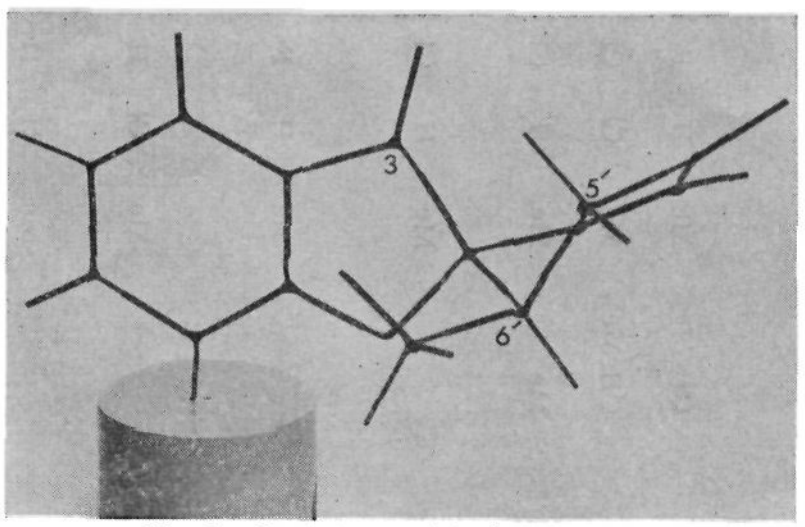


Fig. 2.—Dreiding model of griseofulvin.

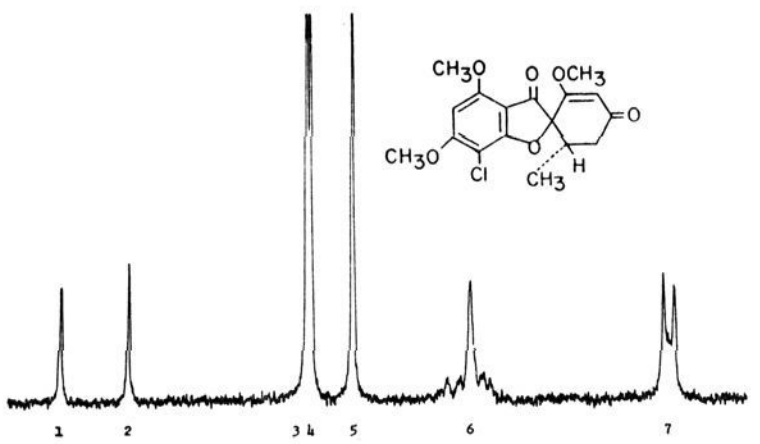


Fig. 3.—N.m.r. spectrum of epigriseofulvin (5% in $CDCl_3$).

Band	τ Value[a]	Functionality
1	3.85	Aromatic H
2	4.42	Vinyl H
3	5.97	Aromatic OCH_3
4	6.00	
5	6.38	Vinyl OCH_3
6	7.51(m)	CH_2–CH
7	9.10 (J = 6 c.p.s.)	C—CH_3

[a] Tetramethylsilane = 10.0.

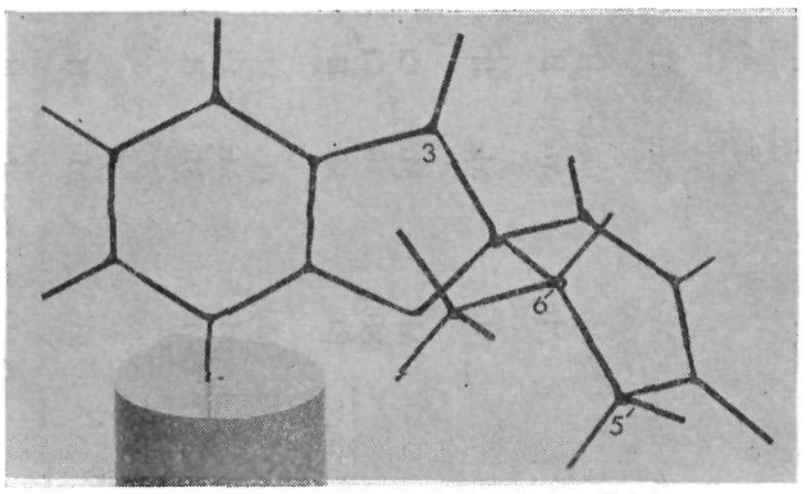


Fig. 4.—Dreiding model of epigriseofulvin.

For the most part, the assignments followed directly from the chemical shift, area and spin-coupling parameters. Where ambiguities existed, as with the methoxyl groups, the correct assignments were arrived at by an analysis of the changes induced by structural variants. In this connection, the spectra of the 4′-ethoxy and 4′-isopropoxy analogs of isogriseofulvin provided two key clues: (1) disappearance of the peak between 6.3 and 6.4 τ, which clearly linked this resonance with the 2′-methoxyl; (2) pronounced upfield shift of one of the aromatic methoxyl signals, an effect that probably arises from long range shielding of the 4-methoxyl by the 2′-ketone. Evidence that the 4-rather than the 6-methoxyl was implicated in this process came from a comparison of 5-chlorogriseofulvin and 5-chloro-7-de-

(1) (a) C. H. Kuo, R. D. Hoffsommer, H. L. Slates, D. Taub and N. L. Wendler, *Chemistry & Industry*, 1627 (1960); (b) D. Taub, C. H. Kuo and N. L. Wendler, *ibid.*, 557 (1962); (c) D. Laub, C. H. Kuo and N. L. Wendler, *ibid.*, 1617 (1962); (d) D. Taub and N. L. Wendler, *Angew. Chem.*, **74**, 586 (1962); (e) D. Taub, C. H. Kuo, H. L. Slates and N. L. Wendler, *Tetrahedron*, Dec., 1962.

(2) Subsequent to the submission of this paper for publication there has appeared a paper on the synthesis of griseofulvin analogs by M. Gerecke, E. Kyburz, C. v. Planta and A. Brossi, *Helv. Chim. Acta.*, **45**, 2241 (1962), which includes some n.m.r. data.

TABLE I

Compound	R	R_1	X_1	X_2	X_3	5 and/or 7-H	3'-H and 5'-H	4- and 6-CH_3O	2'-CH_3O or 4'-CH_3O	$-CH_2CH-$[a]	6'-CH_3[a]	Miscellaneous[a]
						Griseofulvins (I)						
Griseofulvin[a]	Me	Me	H	Cl	H	3.85	4.49	5.96 6.01	6.37	~7.3m	9.02	
(Acetone-d_6)						3.42	4.42	5.87 5.99	6.29	~7.3m	9.05d	
Epigriseofulvin[a,l]	Me	OMe	H	Cl	H	3.85	4.42	5.97 6.00	6.38	7.41m	9.10d	
7-Fluoro-7-dechloro[b]	Me	Me	H	F	H	3.86		5.98 6.06	6.39	~7.3m	9.02d	
						3.95	4.46					
(±)-Dechloro[c]	Me	Me	H	H	H	3.77 3.96	4.50	6.10	6.39	~7.3m	9.04d	
						3.80 4.00						
5-Chloro[c]	Me	Me	Cl	Cl	H		4.42	5.83 5.92	6.34	~7.3m	9.00d	
3',5-Dichloro[c]	Me	Me	Cl	Cl	Cl			5.82 5.92	5.97	~7.2m	9.03d	
2'-Ethoxy[a]	Et	Me	H	Cl	H	3.87	4.47	5.96 6.02		~7.3m	9.07d	6.16q $-CH_2O-$ 8.81t CH_3C
(±)-6'-Desmethyl[d]	Me	H	H	Cl	H	3.86	4.41	5.98 6.02	6.35	7.45m–7.67m		
(±)-5-Chloro-7-dechloro[c]	Me	Me	Cl	H	H	3.54	4.41	5.84 5.99	6.36	~7.3m	9.04d	
3'-Chloro[c]	Me	Me	H	Cl	Cl	3.83		5.93 6.00	6.00	~7.2m	9.00d	
						Isogriseofulvins (II)						
4'-Ethoxy[a]	Et	Me	H	Cl	H	3.92	4.60	6.00 6.10		~7.2m	8.97d	6.04q $-CH_2O-$ 8.61t CH_3C
Isogriseofulvin[a]	Me	Me	H	Cl	H	3.93	4.58	5.99 6.10	6.22	~7.2m	8.96d	
4'-Isopropoxy[f]	$CH(Me)_2$	Me	H	Cl	H	3.95	4.60	6.00 6.10		~7.2m	8.99d	5.50m >CHO– 8.66d $(CH_3)_2C$
4'-Isopropoxy-6-hydroxy[b]	$CH(Me)_2$	H	H	Cl	H	4.45	4.45	6.33		~7.2m	9.00d	0.47 OH 5.43m >CHO 8.62d $(CH_3)_2C$
(Acetone-d_6)						3.75	4.62	6.29		~7.2m	9.02d	5.36m; 8.64d
4'-Isopropoxy-7-fluoro-7-dechloro[g]	$CH(Me)_2$	Me	H	F	H	3.90	4.60	6.01 6.12		~7.2m	8.95d	5.60m >CHO 8.65d $(CH_3)_2C$
						3.95						
4'-Isopropoxy-3',5-dichloro[g]	$CH(Me)_2$	Me	Cl	Cl	Cl			5.93 5.96		~7.1m	8.95d	5.17m >CHO 8.55d $(CH_3)_2C$
3'-Chloro[f]	Me	Me	H	Cl	Cl	3.96		5.96 6.15	5.96	~7.1m	8.91d	
						Dehydrogriseofulvins (III)[o]						
Dehydrogriseofulvin[h,i,k]		Me	H	Cl		3.85	3.85 (5'-H)	5.96 6.02	6.37		8.20	
							4.41 (3'-H)				8.23	
(Acetone-d_6)						3.38	3.82 (5'-H)	5.83 6.00	6.27		8.19	
							4.30 (3'-H)				8.22	
(±)-7-Fluoro-7-dechloro[b]		Me	H	F		3.39	3.84 (5'-H)	5.89 6.01	6.26		8.18	
											8.21	
(Acetone-d_6)						3.49	4.29 (3'-H)					
							4.32					
(±)-5-Chloro-7-dechloro[c]		Me	Cl	H		3.48	3.71 (5'-H)	5.87 6.00	6.36		8.20	
							4.35 (3'H)				8.23	
							4.38					
(±)-Dechloro[c]		Me	H	H		3.75	3.87 (5'-H)	6.10	6.35		8.20	
						3.98	4.42 (3'-H)				8.22	

chlorogriseofulvin with the 3',5-dichloro-4'-isopropoxy analog of isogriseofulvin. The unambiguously assigned 4-methoxyl resonance at 5.83 τ was displaced 0.13 τ upfield upon 4'-isopropoxylation; and when this incremental shift was applied to the pair griseofulvin *vs.* 4'-isopropoxy analog of isogriseofulvin, it was apparent that the 5.96 τ peak is strongly preferred as that associated with the 4-methoxyl group.

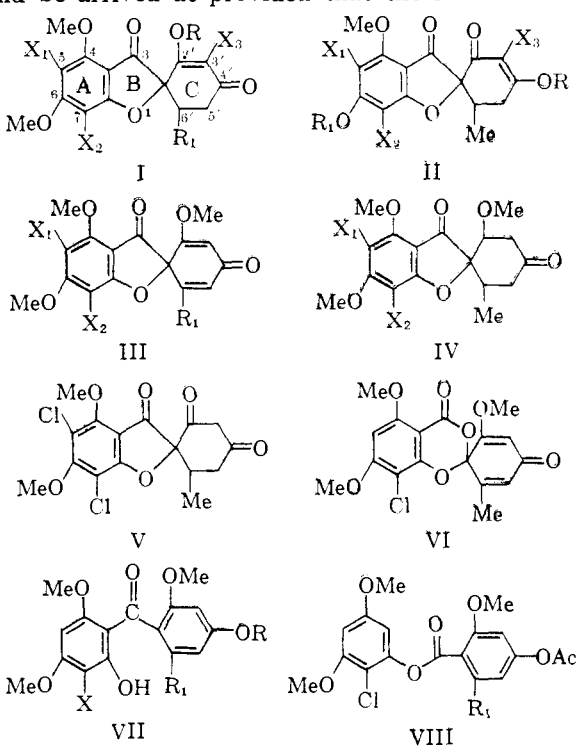
The usefulness of long range interactions as a source of intimate structural information was well illustrated

(±)-6′-Desmethyl[d]	H	H	Cl		3.83	3.62 (5 + 6)	5.96	6.01	6.32			
						4.26 (3′-H)						
5-Chloro-7-dechloro[e]	Me	Cl	H		3.15	3.83 (5′-H)		5.85	6.22		8.24	
(Acetone-d_6)						4.28 (3′-H)						
Dihydrogriseofulvins (IV)												
Dihydrogriseofulvin[a,i]		H	Cl	3.88			5.99	6.03	6.71	~7.3m	9.08d	6.1m
(±)-Dechloro[c]		H	H	3.82								
				3.85			6.11	6.13	6.73	~7.4m	9.08d	6.1m
				4.00								
				4.03								
(±)-7-Fluoro-7-dechloro[b]		H	F	3.88			5.99	6.05	6.71	~7.3m	9.08d	6.1m
				3.96								
5-Chloro-7-dechloro[c]		Cl	H	3.65			5.84	6.00	6.70		9.09d	
(Acetone-d_6)				3.30			5.87	5.93	6.70		9.10d	
Miscellaneous												
						4.52	5.92	5.98		~7.1m	9.00d	
5-Chlorogriseofulvic acid[e] (V)						3.94 (5′-H)		6.01	6.38		7.90	
Griscolactone[j] (VI)				3.77		4.10 (3′-H)					7.93	

[a] A. E. Oxford, H. Raistrick and P. Simonart, *Biochem. J.*, **33**, 240 (1939); J. F. Grove, J. MacMillan, T. P. C. Mulholland and M. A. T. Rogers, *J. Chem. Soc.*, 3949, 3977 (1952). [b] Ref. 3. [c] Ref. 1b. [d] Ref. 1c. [e] T. Walker, W. K. Warburton and G. B. Webb, *J. Chem. Soc.*, 1277 (1962). [f] L. A. Duncanson, J. F. Grove and P. W. Jeffs, *ibid.*, 2929 (1958). [g] C. H. Kuo, unpublished. [h] A. I. Scott, *Chemistry & Industry*, 195 (1958); A. C. Day, J. Nabney and A. I. Scott, *J. Chem. Soc.*, 4067 (1961). [i] T. P. C. Mulholland, *ibid.*, 3987 (1952). [j] Ref. 1e. [k] Ref. 1a. [l] J. F. Grove, J. MacMillan, T. P. C. Mulholland and J. Zealley, *J. Chem. Soc.*, 3967 (1952). [m] L. A. Duncanson, J. F. Grove, J. MacMillan and T. P. C. Mulholland, *ibid.*, 3555 (1957). [n] The symbols d, t, q and m signify the spin–spin character as doublet, triplet, quartet and multiplet where all components are not clearly visible. [o] In all cases, except for the (*l*)-6′-Desmethyl analog, long range coupling of about 1.5 c.p.s. between the 3′ and 5′ protons was observed.

also in the benzophenone series where all *ortho* substituents sustained upfield shifts by virtue of neighboring ring anisotropy. The detectability of this anisotropic effect itself can be taken as good evidence that the aromatic rings are skewed, a conclusion in agreement with a recent study.[2a] In the analogous phenyl benzoates, where ring separation is increased, the delicate configurational and proximity requirements necessary for the long range shielding are destroyed with the consequence that the *ortho* substituents exhibit unperturbed chemical shifts.

While configurational assignments for the 6′-methyl group of griseofulvin[3] and epigriseofulvin could not be readily inferred from the spectra (Fig. 1 and 3), a decision could be arrived at provided that the reasonable assumption is made that an equatorial methyl on a half-chair C-ring is predominant in both isomers. This is based on an analysis of the $-CH_2CH-$ patterns which are strikingly dissimilar in breadth. The observed lines in griseofulvin cover a relatively broad region (close to 60 c.p.s.), which is probably due to a large chemical shift between the 5′-protons. This is in marked contrast to *epi* isomer where the near coincidence of these resonances indicates a corresponding near equivalence of the 5′-protons. Dreiding models (Fig. 2 and 4) reveal that the 5′-β-proton is located close to the 3-ketone in the 6′β-methyl structure[4] and is, moreover, suitably positioned to experience a paramagnetic deshielding. Under these conditions, one might anticipate a distinct chemical shift between the methylenic protons, or at least a larger shift than for the 6′α-methyl isomer where no alternate proposal for non-equivalence appears possible. This view is reinforced by the observation that in all other respects, particularly with regard to the groups at 4′ and 6′, the environments of the two methylene proton pairs are inappreciably different. This line of reasoning thus favors associating a 6′β-methyl with that isomer having the more non-equivalent 5′-protons, *i.e.*, griseofulvin.



A number of compounds, run both in $CDCl_3$ and acetone-d_6, revealed only minor solvent effects except for the aromatic protons which were persistently shifted downfield in acetone by about 0.4 τ. In another instance, the anomalous nature of the 6-hydroxy-4′-iso-

(2a) J. R. Merrill, *J. Phys. Chem.*, **65**, 2023 (1961).

(3) For the assignment of the stereochemistry in the griseofulvin series see: J. MacMillan, *J. Chem. Soc.*, 1823 (1959).

(4) The prefix β signifies group disposition above the plane of ring C vicinal to the C$_3$-carbonyl group. Correspondingly, α designates group orientation below this same plane.

TABLE II[a]

Compound	X	R	R_1	Aromatic H		CH_3O	CH_3—C—	OH	$CH_3C(=O)O$	—C(=O)H
Benzophenones (VII)										
2′,4,6-Trimethoxy-4′,2-dihydroxy-6′-methyl-3-chloro[h,i,k]	Cl	H	Me	3.78	3′ + 5′	6.07		−4.35		
				4.07	5	6.38	7.91	6.13		
						6.54				
(Acetone-d_6)				3.63	3′ + 5′	5.99				
				3.70	5	6.35	7.90	1.45		
						6.43				
2′,4,6-Trimethoxy-4′,2-dihydroxy-6′-methyl-3-fluoro[b]	F	H	Me	3.60	3′ + 5′	5.97	7.87	−3.65		
				3.66, 3.76	5	6.32		1.58		
(Acetone-d_6)						6.45				
2′,4,6-Trimethoxy-4′-acetoxy-2-hydroxy-6′-methyl-3-fluoro[b]	F	Ac	Me	3.50	3′ + 5′	6.05	7.80	−3.47	7.65	
				4.12, 4.21	5	6.31				
						6.59				
2′,4,6-Trimethoxy-4′,2-dihydroxy-6′-methyl[e]	H	H	Me	3.80	3′ + 5′	6.22	7.93	−3.90		
				3.89(d)		6.44		3.98		
				4.21(d)		6.64				
2′,4,6-Trimethoxy-4′,2-dihydroxy-3-chloro[d]	Cl	H	H	2.65, 2.80	6′	6.00				
				3.41, 3.56	5′	6.34		6.27		
				3.49	3′	6.38				
				3.64	5					
Phenyl benzoates (VIII)										
2′,3,5-Trimethoxy-4′-acetoxy-6′-methyl-2-chloro[j,k]			Me	3.40	3′ + 5′	6.12	7.42		7.61	
				3.57	4 + 6	6.14				
						6.18				
2′,3,5-Trimethoxy-4′-acetoxy-2-chloro[d]			H	1.74, 1.90	6′	6.11				
				3.18	3′ + 5′	6.14			7.71	
				3.57	4 + 6	6.22				
Phenols and related compounds										
1,2,4-Trimethoxybenzene				3.10, 3.25	6	6.16				
						6.18				
				3.46, 3.50	3	6.24				
				3.55, 3.62	5					
1,2,3-Trimethoxybenzene				2.80, 2.92, 2.97	5					
				3.08		6.16				
				3.35, 3.49, 3.52	4 + 6					
3-Methoxy-5-hydroxyphenol (Acetone-d_6)				4.02	(all)	6.28		1.85		
3,5-Dimethoxyphenol				3.94	(all)	6.28		4.26		
3-Chloro-3,5-dimethoxyphenol[l]				3.75		6.16				
				3.80				4.29		
				3.88, 3.92		6.25				
4-Chloro-3,5-dimethoxyphenol[l]				3.85		6.19		4.64		
6-Acetyl-2-chloro-3,5-dimethoxyphenol[m]				3.57		5.94		−4.43	7.37	
(Acetone-d_6)				4.00		6.04		−4.40	7.38	
($CDCl_3$)						6.06				
6-Formyl-2-chloro-3,5-dimethoxyphenol[l]				4.00		6.04		−2.80		−0.11
						6.10				

[a] For references, see Table I.

propoxy analog of isogriseofulvin was indicated both by the large C_5-proton shift (0.7 τ) as well as the atypical high field positions of all protons associated with the aromatic ring. These upfield shifts possibly have their explanation in the fact that the aromatic moiety, being a *p*-hydroxyacetophenone type, may have a considerable amount of tautomeric dienone character. The alternate view, that the increased shielding is a normal consequence of 6-demethylation, appears difficult to support on theoretical grounds.

As a technique for discriminating between the griseofulvin and isogriseofulvin skeletons, n.m.r. proved quite powerful. Its broad range of applicability stems directly from the extensive, characteristic shielding changes that accompany this transformation, the most useful of which are listed in Table III. These τ values are average positions calculated from most of the available data, but do not include cases where a proton shift is obviously perturbed by functionality not characteristic of the parent molecules. Also excluded was 3′-

chloroisogriseofulvin where the atypical 4-methoxy and 6′-methyl resonances hint at some minor, but nonetheless significant, environmental change.

In assessing the general character of the data, it is apparent that the clear delineation of all proton types together with the sensitivity of the chemical shifts toward structural change enables n.m.r. to play a very important role in investigating the griseofulvin species. Changes in functionality influence the shielding values

TABLE III

Proton type	τ^a (Griseofulvins)	τ^a (Isogriseofulvins)	$\Delta\tau$
4-CH_3O	5.97	6.10	+0.13
5-H	3.86	3.94	+ .08
3′-H	4.48	4.60	+ .12
6′-CH_3	9.02	8.96	− .06

[a] Average deviation for all values ±0.02 or less.

of nearby protons in a characteristic fashion. Furthermore, when more than one functional group is introduced, the individual effects appear to be additive. These two features are of obvious utility and allow the setting up of a list of incremental shift values reminiscent of the work in steroids.[5,6]

Although most of the spectra could be interpreted satisfactorily in terms of the foregoing simple rules, there were several atypical effects. The cases of 3′-chloroisogriseofulvin and the 6-hydroxy-4′-isopropoxy analog of isogriseofulvin have already been mentioned. Other anomalies were: (1) the selective downfield shifts of the 6′-methyl and 3′-olefinic proton in griseolactone (VI), (2) the collapse of the long range C_3'–C_5' proton coupling in 6′-desmethyldehydrogriseofulvin and (3) the perturbation of the $-CH_2CH-$ pattern by 5-chlorination.

The last example serves as a reminder that a remote functionality is not always without influence. It is reasonable to suppose that the effect is an indirect one, arising from a change in ring or possibly 3-keto anisotropy induced by the 5-chloro group.

The proton magnetic resonance data were obtained with a 60 megacycle Varian Associates Model 4300B spectrometer. Unless otherwise stipulated, spectra were run as dilute solutions (5% or less) in $CDCl_3$. The resonance positions were determined relative to an external benzene reference and scaled by the usual side band method.[7] The shielding numbers were calculated from the equation[8] $T = \Delta\gamma/\gamma_0 + 3.60$, where $\Delta\gamma$ is the observed displacement from benzene in cycles per second, and γ_0 is the spectrometer frequency in megacycles. For acetone, 2.85 was used as the constant in place of 3.60. The precision of the chemical shifts and coupling constants is approximately ±1 cycle.

(5) J. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).
(6) N. R. Trenner, B. H. Arison, D. Taub and N. L. Wendler, *Proc. Chem. Soc.*, 214 (1961).
(7) J. T. Arnold and M. E. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).
(8) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

Application of Mass Spectrometry to Structure Problems. X.[1] Alkaloids of the Bark of *Aspidosperma quebracho blanco*[2]

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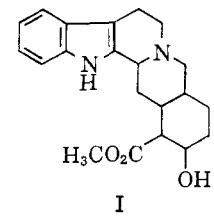
A detailed investigation, with the aid of gas chromatography and mass spectrometry, of the crude alkaloid mixture obtained from the bark of *Aspidosperma quebracho blanco* led to the detection of over twenty compounds. Sixteen of these were isolated in quantities sufficient for the determination of their structure. Three were those known to occur in this plant yohimbine (I), aspidospermine (II) and quebrachamine (III), while the remaining ones turned out to be related to II or to belong to a new group of which aspidospermatine (338B) is the most abundant derivative. The interpretation of the mass spectra which made it possible to arrive at the structures of these alkaloids is discussion in detail.

Investigations of the alkaloids of the bark of *Aspidosperma quebracho blanco* Schlecht. during the last century led to the isolation[3] of six alkaloids, aspidospermine, quebrachamine, quebrachine, aspidospermatine, hypobrachine and aspidosamine. Of these, quebrachine was later found[4] to be identical with yohimbine (I) and only recently the structures of two others, aspidospermine (II)[5] and quebrachamine (III),[6] were established. The remaining three alkaloids were not well characterized and had not attracted any serious attention since their isolation.[3] In spite of the great interest which the Apocynaceae family in general and the genus Aspidosperma in particular received during the last decade,[7] *A. quebracho blanco* has not been reinvestigated by more modern techniques although the plant material is readily available. An exception is a recent short note reporting the isolation of a new glucoalkaloid, quebrachacidin.[8]



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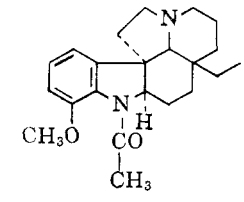
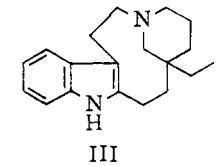


II



III

Recently we have shown that mass spectrometry can be used advantageously for the determination of the structure of alkaloids.[9a,b] This technique was also used for the determination of the structure of quebrachamine (III)[6] which necessitated the determi-

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(5) J. F. D. Mills and S. C. Nyburg, *Tetrahedron Letters*, No. **11**, 1 (1959); H. Conroy, P. R. Brook and Y. Amiel, *ibid.*, No. **11**, 4 (1959).
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(9) (a) K. Biemann, *Tetrahedron Letters*, No. **16**, 9 (1960); (b) For a detailed discussion of this subject see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 8.