mesylate **25:** IR ($CHCl_3$) 1770 cm^{-1}; NMR ($CDCl_3$) δ 3.07 (s, 3 H), 0.96 (d, 3 H, J = 7 Hz).

Preparation of Intermediate 26. A solution of the above tetrahydropyranyl ether **25** (30 mg, 0.07 mmol) in 2.0 mL of absolute methanol containing 7 mg of *p*-toluenesulfonic acid was allowed to stir for 30 min at 0 °C. After an additional 45 min at room temperature, the solvent was evaporated under reduced pressure. The crude alcohol was purified on 12 g of SilicAR CC-7 using ether–benzene, 2:1. There was obtained 27 mg (99%) of alcohol [IR ($CHCl_3$) 3530, 2765 cm^{-1}; NMR ($CDCl_3$) δ 4.86 (m, 1 H), 4.45 (d, . 2 H, J = 4 Hz), 3.62 (br s, 2 H), 3.05 (s, 3 H), 1.75 (s, 3 H), 0.95 (d, 3 H, J = 7 Hz)] which was used directly in the next reaction.

A mixture of the above alcohol (18 mg, 0.05 mmol) and 222 μL of 0.7 M Jones reagent in 1.2 mL of acetone was allowed to stir at 0 °C for 1.5 h. The reaction was quenched by the addition of 2-propanol. After evaporation of the solvent in vacuo, the residue was taken up in ethyl acetate.[19] The resulting crude carboxylic acid was esterified with an ethereal solution of diazomethane. Chromatography of the crude ester on SilicAR CC-7 using ether–benzene, 1:2, provided 20 mg (100%) of pure **26:** IR (CCl_4) 1740, 1778 cm^{-1}; NMR (CCl_4) δ 4.68 (m, 1 H), 4.38 (d, 2 H, J = 4 Hz), 3.62 (s, 3 H), 2.98 (s, 3 H), 1.74 (s, 3 H), 0.97 (d, 3 H, J = 7 Hz).

(±)-Ivangulin (3). A solution of 17 mg (0.04 mmol) of mesylate **26** in 1.0 mL of dry benzene containing 20 μL of 1,5-diazabicyclo-[5.4.0]undec-5-ene was allowed to stir at room temperature for 30 min. The reaction mixture was purified directly on 6.0 g of silica gel. Elution with ether–hexanes (1:2) gave 12.5 mg (99%) of crystalline (±)-ivangulin, mp 66–66.5 °C [IR($CHCl_3$) 3020, 2960, 2925, 2875, 2845, 1738, 1730, 1660, 1620, 1460, 1438, 1405, 1382, 1368, 1355, 1328, 1272, 1175, 1145, 1110, 1080, 1038, 1005, 989, 970, 948, 905, 865, 815 cm^{-1}; NMR ($CDCl_3$) δ 6.27 (d, 1 H, J = 3 Hz), 5.68 (d, 1 H, J = 3 Hz), 4.87 (q, 1 H, J = 5 Hz), 3.64 (s, 3 H), 3.25 (m, 1 H), 1.70 (s, 3 H), 0.94 (d, 3 H, J = 7 Hz)] whose NMR and IR spectra were in complete accord with spectra provided by Professor W. Herz.

Acknowledgments. This investigation was supported by a Public Health Service Research Grant (CA 13689-05) from the National Cancer Institute and the National Institutes of Health NMR Facility for Biomedical Studies (RR-00292). We thank Messrs. V. Bell and G. Herman for mass spectral data. We are grateful to Professor Werner Herz for providing us with the NMR and IR spectra of natural ivangulin.

Registry No.—**3,** 63640-50-6; **4,** 63600-03-3; **5,** 63600-04-4; **5** tosylhydrazone, 63600-05-5; **6,** 63600-06-6; **7,** 63600-07-7; **8,** 63600-08-8; **9,** 63600-09-9; **10,** 63600-10-2; **11,** 63600-11-3; 11 free ketone, 63600-12-4; **12,** 63600-13-5; **16,** 63600-14-6; **17,** 63600-15-7; **18,** 63600-16-8; **19,** 63600-17-9; **22,** 63609-71-2; **23,** 63600-18-0; **23** free alcohol, 63600-19-1; **24,** 63600-20-4; **24** hydroxymethylated product, 63600-21-5; **25,** 63600-22-6; **25** ditetrahydropyran analogue, 63600-23-7; **26,** 63600-24-8; **26** free acid, 63600-25-9; benzyl bromide, 100-39-0.

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Natural Products of Marine Sponges. 7. The Constitution of Weakly Basic Guanidine Compounds, Dibromophakellin and Monobromophakellin

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The isolation and elucidation of the structure of dibromophakellin and monobromophakellin are reported. Although these molecules contain a guanidine moiety in their skeleton, they do not exhibit the high basicity expected from the presence of this functionality. A theoretically plausible explanation for the anomaly in the base strength of these compounds is discussed.

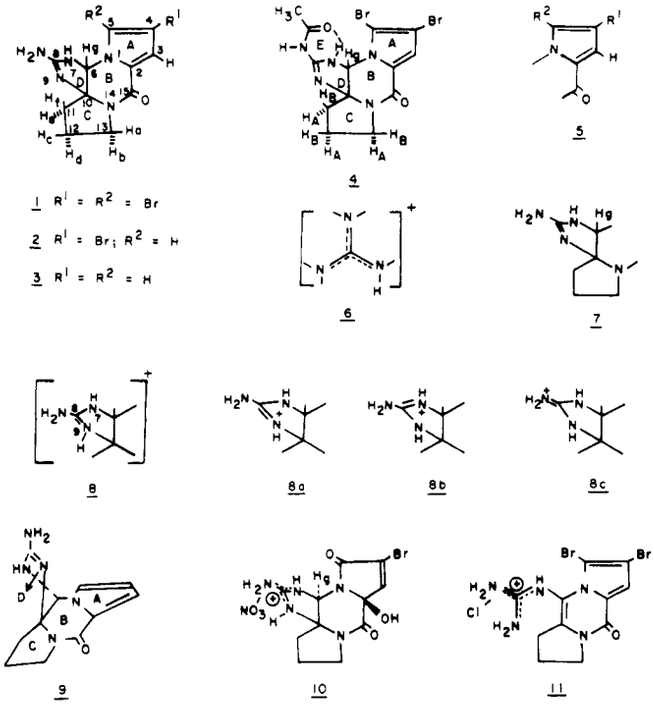
A few years ago we isolated two guanidine derivatives, dibromophakellin and monobromophakellin, from the marine sponge *Phakellia flabellata.*[2] These compounds showed p*K*a values of <8 which were rather low when compared with the p*K*a values of >13.4 reported for other guanidines. This paper describes in detail the isolation and characterization of bromophakellins and discusses the factors which make these compounds behave as weak bases.[3]

Dibromophakellin and monobromophakellin occur as hydrochlorides in the sponge *P. flabellata.* The hydrochlorides exhibit a very mild antibacterial action against *B. subtilis* and *E. coli.* The strong antibacterial activity of the methanol extract of the sponge is due to the presence of some other substance(s) which could not be isolated in pure form.

The sponge showed considerable seasonal variations in the production of monobromophakellin, dibromophakellin, and

the antibacterial substance. The material collected during the summer showed very weak antibacterial activity, and systematic fractionation of these specimens gave only monobromophakellin hydrochloride. In contrast, the specimens collected during the winter were found to be very rich in natural products, and from this material dibromophakellin hydrochloride, a fraction containing a strong antibacterial substance, and several other compounds were isolated.[4] Some specimens collected during the winter were found to contain both dibromophakellin hydrochloride and monobromophakellin hydrochloride.

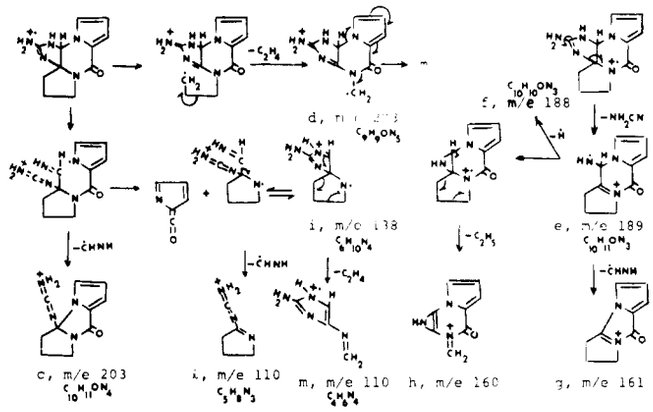
Addition of concentrated ammonia to the aqueous solutions of dibromophakellin hydrochloride ($C_{11}H_{11}N_5OBr_2$·HCl, $[\alpha]^{25}_D$ −203) and monobromophakellin hydrochloride ($C_{11}H_{12}N_5OBr$·HCl, $[\alpha]^{25}_D$ −123) gave the corresponding free bases **1** and **2**. Catalytic hydrogenation of **1** and **2** gave phakellin **3** ($C_{11}H_{13}N_5O$). The UV spectra of the three phakellins showed absorbtion maxima around 233 and 281 nm which were suggestive of the presence of a pyrrole ring having a carbonyl function at the 2 position.[5] The infrared spectra of **1**, **2**, and **3** revealed the presence of amino groups, methylenes, an amide function, and a C=N unit, and provided further support for the presence of a pyrrole ring.



The ^{1}H NMR spectrum of **3** revealed the presence of a $-CH_2CH_2CH_2NCO-$ unit, a highly deshielded methine proton Hg, a 1,2-disubstituted pyrrole ring, and three D_2O-exchangeable protons in the structural framework of phakellins. A comparison of the NMR spectra of **1** and **3** suggested that the two bromine atoms in **1** are present at the 4 and 5 positions of the pyrrole ring. The single bromine atom in **2** was placed at the 4 position because in the NMR spectrum of this compound the two heteroaromatic protons showed doublets (J = 1.8 Hz).[6]

Although both dibromophakellin and monobromophakellin are levorotatory, their molecular rotations differed by more than 400 units and their long-wavelength UV bands showed Cotton effects of the opposite sign. These compounds were shown to possess the same configuration at the asymmetric centers by reacting monobromophakellin with 1 mol equiv of bromine and establishing the identity of the resulting product with dibromophakellin. Since addition of substituents to the pyrrole ring produces vast changes in chiroptic properties of

Scheme I



phakellins, it was concluded that this ring is directly linked to one of the two chiral centers of these molecules.

The mass spectra of **1**, **2**, and **3** were notable for the presence of fragment ion peaks produced by the expulsion of NH_3, HCNH, $CH_2{=}CH_2$, NH_2CN, and the pyrrole ring from M^+. The mechanisms of some of the fragmentation processes are proposed in Scheme I. The peaks at m/e 138 and 110 (doublet) shift cleanly to m/e 141, 113, and 112 in the spectrum of phakellin-d_3.[7] The elemental composition of all ions indicated in Scheme I were established by high-resolution mass measurements. The main significance of mass spectral data is that it provides strong evidence for the presence of a guanidine moiety and structural unit **7** in phakellins.

Partial structures **5** and **7** account for all the atoms of phakellins. These structural units were combined to form a tetracyclic skeleton, and dibromophakellin, monobromophakellin, and phakellin were considered to possess structures **1**, **2**, and **3**, respectively. In these structures, the guanidine double bond was placed at the endocyclic 8,9 position to explain the deshielding of the pyrrolidine proton H_a upon conversion of phakellins to their hydrochlorides. With the double bond at this position, **1**, **2**, and **3** will protonate at N(9). The deshielding of H_a in phakellin hydrochlorides may then be attributed to the reduction in the long-range shielding effect of the lone pair associated with N(9).

The ^{13}C NMR spectrum of monobromophakellin hydrochloride, determined in Me_2SO-d_6, was consistent with the general structure proposed for phakellins. The spectrum showed peaks at δ 156.21 [C(15)], 154.5 [C(8)], 123.81 [C(4)], 122.31 [C(3)], 113.51 [C(5)], 98.29 [C(2)], 82.19 [C(10)], 68.23 [C(6)], 45.24 [C(13)], 37.73 [C(11)], and 19.51 [C(12)]. The chemical shift of C(6) is consistent with the view that this carbon atom is directly linked to two electron-withdrawing groups, the guanidine moiety and the pyrrole nitrogen.

Confirmatory evidence for the tetracyclic skeleton of phakellins was provided by the single crystal x-ray analysis of monoacetyldibromophakellin **4**.[8] The structure was solved by conventional heavy-atom methods. The ORTEP diagram of one molecule of **4** as viewed along the y axis is shown in Figure 1. The coordinates and temperature factors of all atoms found in the electron-density maps, together with their estimated standard deviations (except for the temperature factors in hydrogens), are listed in Table I (Supplementary Material). The bond distances and bond angles derived from this study are compiled in Table II (Supplementary Material). The distances have an esd of the order of 0.01 Å, and the angles of about 0.5°. The equations for the least-squares planes of important structural moieties are given in Table III (Supplementary Material).

The skeleton of phakellins as revealed by the x-ray analysis of **4** is in complete accord with the one proposed on the basis of spectroscopic data and chemical studies. The crystallographic data indicate that the atoms defining the pyrrole ring

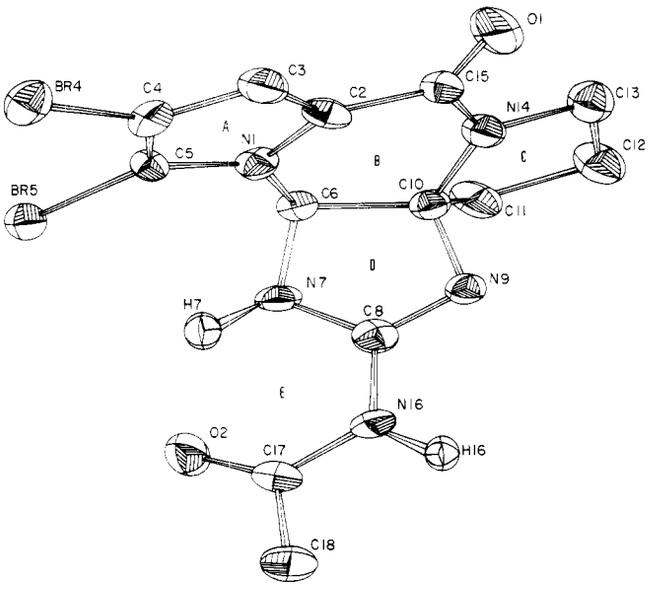


Figure 1. Perspective ORTEP diagram of monoacetyldibromophakellin. The thermal vibration ellipsoids are shown on a 50% probability scale. All hydrogens except H(7) were omitted. The inclusion of H(7) illustrates the hydrogen bonding resulting in the formation of ring E.

of phakellins are coplanar with maximum deviation from the least-squares plane of 0.008 Å. The six-membered ring B is markedly nonplanar as is the pyrrolidine ring C (Table III, Supplementary Material). In general, bond distances and bond angles of these rings agree very well with the accepted values.

The acetylaminoimidazoline ring D has a twisted conformation. An appreciation of the extent of twist can be obtained by examining the displacements of the five atoms of the ring from the least-squares plane. Whereas atoms N(7) and C(10) are located at a distance of 0.140 and 0.152 Å below the ring plane, the atoms C(6), C(8) and N(9) lie above the average plane and are displaced from it by 0.175, 0.037, and 0.079 Å, respectively. The guanidine moiety of ring D is planar; only the central carbon atom of the CN_3 skeleton deviates slightly (0.018 Å) from the least-squares plane (Table III, Supplementary Material). The bonds linking the guanidine moiety to ring B make a dihedral angle of about 29° with each other.

The C(8)–N(9) bond distance of the imidazoline ring is 1.287 Å, which is in close agreement with C=N distance of 1.27 Å reported in the literature.[9] The C(8)–N(7) and C(8)–N(16) bond lengths of 1.368 and 1.39 Å are within the range expected for a carbon–nitrogen single bond adjacent to a double bond.[9] These parameters suggest that in **4** the guanidine double bond is present at the 8,9 position. It is significant to note that phakellins are the only cyclic guanidines which have a double bond at the endocyclic position and which, upon treatment with acetic anhydride, give derivatives of the endocyclic acetylamino type. All other cyclic guanidines have been found to retain the double bond at the exocyclic position and their acetyl derivatives exist in the exocyclic acetylamino form.[10]

In the acetamide group (CH_3COHN) of **4** the N(16)–C(17) and C(17)–O bond lengths are 1.38 and 1.21 Å. These values are similar to those found in acetamide and *N*-methylacetamide.[9] It seems, therefore, that in the acetylaminoguanidine system of **4** the normal amide resonance which imparts a considerable amount of double-bond character to CN bonds and a single-bond character to CO bonds is not suppressed by the opposite mesomeric effect in the imine group.[11]

The crystal structure of **4** revealed the presence of only two hydrogen bonds, one intramolecular and the other intermolecular. The intramolecular hydrogen bond is between the oxygen atom of the acetyl carbonyl and the NH at position 7 (Figure 1; see also structure **4**). Because of this internal H bond, the hydrogen atom H(7) finds itself a member of the planar six-membered ring E. The intermolecular hydrogen bond is between the carbonyl oxygen of ring B and N(16)–H. The atoms participating in the intermolecular hydrogen bond are 2.6-Å apart and the N(16)–H. . .0 angle is 160°. Apart from this, there are no other short contacts in the crystal structure of **4**.

With the availability of a complete x-ray analysis of **4**, the structures assigned to phakellins may be considered to have been fully established. However, an explanation for the low basicity of the guanidine group of these molecules is needed.

In order to explain the anomaly in the base strength of phakellins, it is essential to first consider the reason for the high basicity of guanidines. Guanidines are strong bases because they protonate at the imine nitrogen to give cations which have a resonance-stabilized structure **6**.[12] The extra resonance stabilization of the ions has been estimated to be of the magnitude of 6–8 kcal/mol which would increase the base strength of guanidines very greatly. Resonance in the guanidinium ion has been confirmed by IR spectroscopy, Raman spectroscopy, and x-ray crystallography.[13]

The evidence presented below suggests that phakellins also protonate at the imine nitrogen [N(9)] of the guanidine moiety, but resonance in the resulting cations (hereafter called phakellinium cations) is inhibited. The inhibition of resonance will reduce the tendency of the imine nitrogen of the guanidine groups of **1**, **2**, and **3** to add a proton and in consequence these compounds would behave as weak bases.

Information on the site of protonation of phakellins was obtained by analyzing the NMR spectra of phakellinium cations produced by dissolving **1**, **2**, and **3** in trifluoroacetic acid. In these spectra the guanidinium protons exhibit three singlets in the 7–8.7-ppm region. From low to high field the singlets integrate for 1 H, 1 H and 2 H, respectively. This spectral data is consistent with the protonation of phakellins at N(9) to give cation **8**. The lowest field singlet is assigned to N(7)–H because this resonance is shielded by the anisotropy of the bromine atom present at the 5 position of 4,5-dibromophakellinium cation. It is noteworthy that the vicinal protons of the system H–N(7)–C(6)–Hg do not exhibit spin–spin interactions which is consistent with the dihedral angle of ~90° between N(7)–H and C(6)–Hg bonds. If protonation of phakellins had taken place at N(7), then the dihedral angle between C(6)–Hg and N(7)–H′ [H′ is the second proton on N(7)] would be about 10°. In this case, the resonances of Hg and N(7)–H′ should appear as doublets. Since none of the resonances assigned to the guanidinium protons and Hg showed this feature, phakellins were considered not to protonate at N(7). The degree of charge delocalization in the guanidinium system of phakellinium cations was determined by comparing the IR spectra of guanidine hydrochlorides with those of phakellin hydrochlorides and by studying the exchange of the guanidinium protons in phakellin hydrochlorides with D_2O.

The IR spectra of several guanidine hydrochlorides have been reported in the literature.[14] The data reveal that when a guanidino group protonates the characteristic C=N absorbtion vanishes (due to resonance) and is replaced by two bands in the 1700–1580 cm^{-1} region, which correspond to the antisymmetrical vibrations of the carbon–nitrogen bonds within the guanidinium group. The positive charge on the nitrogen atoms of a resonance-stabilized guanidinium cation is appreciably smaller than in ammonium ions. Consequently,

the NH-stretching modes in the IR spectra of guanidine hydrochlorides occur above 3200 cm^{-1} (i.e. in the region of free amines) rather than below 3200 cm^{-1} where typical amine hydrochlorides normally absorb.

The IR spectra of phakellin hydrochlorides displayed a broad band stretching from 3500 to 2900 cm^{-1} (Figure 2), indicating that in cations the positive charge is not evenly distributed over all the atoms of the CN_3 skeleton. The 1700–1580-cm^{-1} region of the spectra showed one band around 1650 cm^{-1} and a second around 1695 cm^{-1}. The former band is due to the absorption by the amide carbonyl. Since there is no absorption around 1595 cm^{-1}, the band at 1695 cm^{-1} is assigned to the absorption by the C=N group which apparently does not vanish when phakellins are protonated. In the IR spectra of phakellins, the C=N absorption occurs around 1670 cm^{-1}. The increase in C=N stretching frequency upon passing from phakelline to phakellin hydrochlorides suggests that in cations most of the positive charge resides on the imine nitrogen.

In the NMR spectrum of dibromophakellin hydrochloride, the guanidinium protons give two broad bands centered at δ 9.8 [2 H, C=NH and N(7)–H] and 8.2 (2 H, C–NH_2). When the spectrum was recorded immediately after the addition of 1 equiv of D_2O, the 9.8-ppm band was found to have broadened considerably and partially merged with the 8.2-ppm band. The combined intensity of the two bands corresponded to three protons. Addition of 2 equiv of D_2O made the 9.8-ppm band disappear completely within 10 min. The 8.2-ppm band did not start broadening and diminishing in intensity until a total of 6 equiv of D_2O had been added. In fact, 10 equiv of D_2O was needed to make this band disappear completely in 20 min. Assuming exchange rates of protons reflect charge distribution in the CN_3 skeleton, then the NH_2 group of the cations may be considered to bear little, if any, positive charge. This conclusion appears to be valid when it is realized that the in the phakellinium skeleton the NH_2 group is least hindered, but its protons exchange last upon titration with D_2O.

Although the acetyl carbonyl of **4** can conjugate strongly with an exocyclic double bond, this compound could not be converted to the acetylimino form by reacting with an acid or a base. This observation also suggests that the guanidino group of phakellins has very little tendency to develop a $C{=}N^+$ character at the 8,16 position.

The evidence for the presence of some positive charge on N(7) of cations is provided by the large downfield shift (0.5–0.6 ppm) of Hg upon protonation of **1**, **2**, and **3**. If during protonation the guanidinium double had entirely shifted to the 7,8 position, then the resonance of Hg would have been deshielded by 1.5–1.8 ppm (1–1.3 ppm for the double bond plus 0.5 ppm for the positive charge). Based on this assumption, a downfield shift of 0.5 ppm in the resonance of Hg should correspond to about 35% imminium character at the 7,8 position of phakellinium cations.

The data presented above suggests that the phakellinium cations should be represented by a structure to which the three canonical forms **8a**, **8b**, and **8c** make contributions in the following order: **8a** > **8b** ≫ **8c**. Hence, resonance in phakellinium cations is inhibited. Since the guanidine moiety of phakellins is planar, the inhibition of resonance in cations will have to be attributed to some other structural features of the phakellinium skeleton.

It was pointed out earlier that the imidazoline ring (ring D) of phakellins has a twisted conformation, because it is fused to the chair-shaped ring B via two bonds which are skew (see structure **9**). Inspection of the molecular models indicated that ring D cannot be made planar without introducing severe conformational strains in ring B. It may then be argued that perhaps it is the twisted shape of the imidazoline ring which inhibits resonance in phakellinium cations. The mechanism

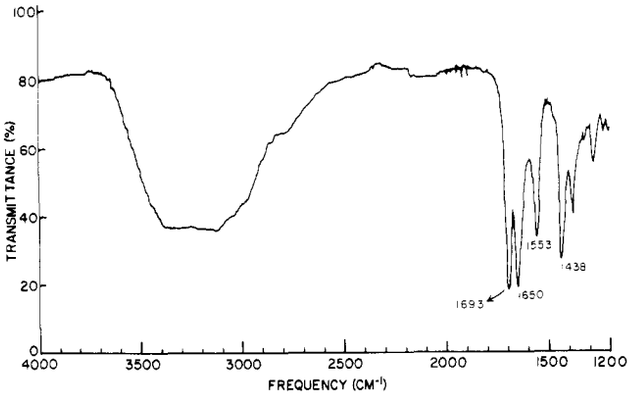


Figure 2. The 4000–1200-cm^{-1} region of the IR spectrum of dibromophakellin hydrochloride determined in KBr.

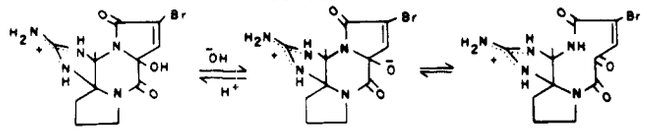
by which the resonance could have been inhibited may be visualized by considering the formation of the guanidinium cation in terms of molecular orbital description. Protonation of the imine nitrogen of a guanidino group first gives a cation which has a positive charge localized on the carbon atom, and the three nitrogen atoms exhibit pyramidal arrangement of valencies.[13] In this species,[13,14] the axis of the p-type lone pairs on the nitrogen atoms would be oriented in a direction not parallel to the vacant orbital of the carbon atom considered as C^+. For resonance to take place, each nitrogen atom of this ion will have to change from pyramidal arrangement of valencies to the planar ones. In the case of phakellinium cations, a simultaneous change in the hybridization of all three nitrogen atoms followed by equal interaction of the three lone pairs of electrons with the vacant p orbital of the central carbon atom may require the imidazoline ring to be planar. Since this ring cannot become planar, the sequence of events leading to resonance will be suppressed and the imine nitrogen of phakellins will exhibit reduced tendency to add a proton. One way to test this hypothesis would be to convert the tetracyclic skeleton of phakellins into a structure in which the imidazoline ring is planar. If the explanation offered for the inhibition of resonance in phakellinium cations is correct, then the guanidine moiety of the transformation product should be highly basic, and upon protonation this functionality should give a resonance-stabilized cation.

Oxidation of dibromophakellin with dilute nitric acid gave a compound ($C_{11}H_{12}N_5O_3Br{\cdot}HNO_3{\cdot}H_2O$) to which structure **10** was assigned on the basis of spectroscopic data and single crystal x-ray analysis.[18] Molecular models revealed that in **10** the six-membered ring is a boat with a planar imidazoline ring strainlessly fused to the eclipsed bonds at the side of the boat. Thus, structure **10** has all the features needed for verifying the explanation offered for the inhibition of resonance in phakellinium cations.

In the IR spectrum of **10** the NH-stretching frequencies occurred above 3200 cm^{-1}, and the 1700–1580-cm^{-1} region contained two bonds (1695 and 1600 cm^{-1}) due to the antisymmetric vibrations of the CN bonds of a resonance-stabilized disubstituted guanidium cation. Thus, unlike the guanidinium group of phakellinium cations, the guanidinium group of **10** has a resonance-stabilized structure.

Potentiometric titrations of **10** revealed that this compound is a monoacidic base of pK_a 7.9. If this pK_a is due to the deprotonation of a guanidinium group, then upon treatment of **10** with sodium hydroxide the corresponding free base should be liberated. When 0.01 N NaOH was added to the aqueous suspension of **10**, a clear solution was obtained. Lyophilization of the solution gave a product which was crystallized from a 90% methanol–water mixture. The results of several combustion analyses indicated that the crystallized material is a

Scheme II



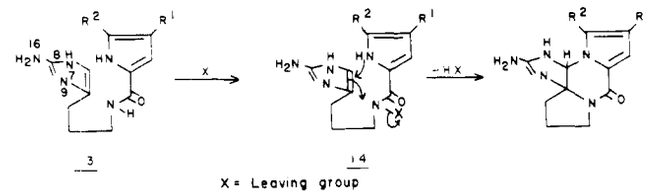
12

free base ($C_{11}H_{12}N_5O_3Br\cdot 2\text{–}3H_2O$) which retains variable amounts of moisture very tenaciously. The 1740–1630-cm^{-1} region of the IR spectrum of the free base showed a broad band with shoulders at 1720, 1690, 1665, and 1640 cm^{-1}. In addition, the spectrum showed peaks at 1600 and 1570 cm^{-1}. The most significant point which emerges from this data is that those bands in the IR spectrum of **10** which must be associated with the guanidinium system appear unchanged in the free base. This observation suggests that the deprotonation of **10** upon treatment with a base takes place at a site other than the guanidinium group. It is proposed that the p*K*a value of **10** as determined by potentiometric titrations represents the deprotonation of the hydroxyl group and formation of ionic species shown in Scheme II.

Reactions of **10** or **12** with dimethyl sulfate or methyl iodide failed to give the corresponding *O*-methyl derivative. Consequently, the normal basic function of **10** could not be masked to determine the exact p*K*a value of the guanidinium group. Nevertheless, the indirect evidence presented above clearly suggests that the guanidinium group of **10** is highly basic and this group upon protonation gives a resonance-stabilized cation. From this the conclusion would follow that once the imidazoline ring of phakellins is made planar then the guanidine moiety of this ring behaves like normal guanidine derivatives. The explanation offered for the low basicities of phakellins, therefore, appears to be correct.

Reaction of dibromophakellin with dilute HCl (10–20%) at 100 °C for 1 h gave a mixture which could not be resolved into its components by chromatography over Sephadex G-10. Repeated crystallization of the mixture from water gave a product which showed a single spot on silica gel plates in a variety of solvent systems. Microanalysis and mass spectrometry (M^+ at 387, 389, and 391; $C_{11}H_{11}N_5OBr_2$) indicated that this compound is a hydrochloride of an organic base isomeric with dibromophakellin. Structure **11** is assigned to this compound on the basis of IR, UV, and NMR spectral data. The reaction of dibromophakellin with hydrochloric acid proceeds in the direction of **11** because cleavage of the N(9)–C(10) bond removes conformational strains in the phakellinium cation.

Dihydrooroidin **13** and phakellins appear to be biogenetically related.[21] Perhaps it is because of the biosynthesis of phakellins from **13** that the double bond in **1, 2,** and **3** is lo-



X = Leaving group

cated at the 8,9 position. Once the tetracyclic skeleton has been formed then migration of the double bond to the exocyclic position will involve a transition state which requires the imidazoline ring to be planar. Since this ring cannot become planar, the guanidine double bond in phakellins and their derivatives is forever locked at the 8,9 position.

In conclusion, it may be stated that the low basicities of phakellins are explainable in terms of I-strain concepts.[15] Theoretically, the basicity of a cyclic guanidine derivative will fall off rapidly as the four atoms of the CN_3 skeleton depart from planarity and/or as the conformational strains in the ring inhibit the nitrogen atoms of the CN_3 skeleton to change from the pyramidal configuration of valencies to planar ones. Since in phakellins the CN_3 skeleton is planar, the lowering of the p*K*a value of the guanidine group of these molecules by as much as 6 p*K*a units may be solely due to the twisted conformation of the imidazoline ring. A rigorous test of the adequacy of this view would require synthesis of the *O*-methyl derivative of **10** and determination of the exact p*K*a of the guanidinium group. Until this is accomplished, the explanation offered for the low basicities of phakellins may be considered to be only partially substantiated. This is especially so when it is realized that a certain amount of hindrance to the protonation of phakellins may also come from the unfavorable interaction between the hydrogen atom C(11)–H_f and the proton to be attached to N(9). If the p*K*a of the guanidinium group of **10** turns out to be greater than that of phakellins but less than that of other guanidine derivatives, then the repulsion term arising from the nonbonded interaction between the hydrogen atoms identified above may also have to be considered.[22]

Experimental Section

Melting points are corrected. Elemental analyses were done by Alfred Bernhardt Microanalytical Laboratories, West Germany. The UV spectra were recorded with a Beckman Model DK-2A ratio recording spectrophotometer. The IR spectra were recorded on a Perkin-Elmer 337 spectrometer. The NMR spectra were recorded on a Varian HR 220-MHz NMR spectrometer.

Dibromophakellin Hydrochloride. The wet sponge (1 kg) and methanol (3 L) were homogenized in a blender to give a fine slurry. After keeping for 48 h at room temperature, the slurry was filtered and the residue extracted twice more with methanol. The methanol from the combined extracts was removed under reduced pressure and the residue was stirred with 400 mL of water for 1 h. The suspension was filtered and the aqueous filtrate was first extracted with three 200-mL portions of ethyl acetate and then four times with water-saturated *n*-butanol, using 200 mL of the solvent each time. The butanol extracts were combined and concentrated under reduced pressure to yield a yellow residue (~12 g). A portion (1.0 g) of this residue was dissolved in a minimum amount of water, and the clear solution (filtered if necessary) was chromatographed on 100 g of Sephadex G-10, packed in a 1 in. i.d. column. By eluting the column with water, 20 25-mL fractions were collected. The fractions (12–15) having an absorbtion maxima at 270–280 and 230–240 nm were combined and lyophilized. The lyophilized material (100 mg) was rechromatographed on 100 g of Sephadex G-10. Elution with water gave fractions which showed UV maxima at 278 and 233 nm. These fractions were combined and freeze-dried to give 85 mg of dibromophakellin hydrochloride. Crystallization from methanol or water gave needles: mp 220–221 °C; $[\alpha]^{25}_D$ −205 (*c* 2.875, MeOH); IR (KBr) 3400–3100, 3100–2600, 1693, 1650, 1553, 1438, 1375, 970 and 740 cm^{-1}; UV (MeOH) 282 (ϵ 9138) and 234 (ϵ 9333). The CD curve showed maxima at 283 ($\Delta\epsilon$ −3.61) and 2 nm ($\Delta\epsilon$ −8.03) and the onset of a third much stronger negative cotton effect with maxima below 210 nm ($\Delta\epsilon$ at 210 nm = 16.06): NMR (Me_2SO-d_6) δ 9.9 (2 H, br, NH and C=NH^+), 8.37 (2 H, br, NH_2), 7.0 (1 H, s, H-3), 6.34 (1 H, s, Hg), 3.65 (1 H, q, H_a, *J* = 18 and 9.5 Hz), 3.43 (1 H, q, H_b, J = 18 and 9.5 Hz), 2.13–2.47 (2 H, m, H_c and H_d), 2.06 (2 H, br, H_e and H_f). In F_3AcOH, the guanidinium protons gave three singlets at δ 8.29 (1 H, NH), 8.18 (1 H, C = NH^+) and 7.11 (2 H, NH_2).

Anal. Calcd for $C_{11}H_{11}N_5OBr_2\cdot HCl$: C, 31.02; H, 2.82; N, 16.45; Br, 37.60; Cl, 8.34. Found: C, 31.05; H, 2.84; N, 16.24; Br, 37.40; Cl, 8.28.

Dibromophakellin (1). Addition of concentrated ammonia to the aqueous solution of dibromophakellin hydrochloride gave a white precipitate which was crystallized from methanol to give pure dibromophakellin as a methanol solvate ($C_{11}H_{11}N_5OBr_2\text{–}CH_3OH$): mp 237–245 °C (dec); p*K*a 7.7; IR (KBr) 3400 (br), 2875, 2950, 1675, 1635, 1587, 1550, 1490, 1437, 972 and 741 cm^{-1}; UV (MeOH) 281 (ϵ 8813) and 233 nm (ϵ 8877); mass spectrum M^+ *m/e* 387, 389, and 391, and fragmentation patterns shown in Scheme I. The CD spectrum displayed maxima at 285 ($\Delta\epsilon$ −4.58) and 239 nm ($\Delta\epsilon$ −12.43) and the onset of another negative cotton effect with maxima below 210 nm ($\Delta\epsilon$ at 210 nm = 26.18): NMR (Me_2SO-d_6) δ 6.81 (1 H, s, H-3), 5.76 (1 H, s, Hg), 4.2 (3 H, br, NH and NH_2), 3.5 (2 H, br, 13-CH_2), 1.85–2.27 (4 H, br m, 11-CH_2 and 12-CH_2).

Anal. Calcd for $C_{11}H_{11}N_5OBr_2\text{–}CH_3OH$: C, 34.43; H, 3.2; N, 16.55;

Br, 40.54. Found: C, 34.29; H, 3.23; N, 16.68; Br, 40.40.

Monobromophakellin Hydrochloride. Monobromophakellin hydrochloride (350 mg) was isolated from the specimens of *P. flabellata* (1 kg) collected during the summer. The isolation procedure was the same as described for dibromophakellin hydrochloride. Monobromophakellin hydrochloride was crystallized from water to give white needles having mp 215–220 °C; $[\alpha]^{25}_D$ −123 (c 3.015, MeOH); IR (KBr) 3100 (br), 1695, 1550, 1475, 930, and 740 cm^{-1}; UV (MeOH) 277 nm (ϵ 5535) and 227 nm (ϵ 7135); CD (MeOH) 275 nm ($\Delta\epsilon$ +2.25), 236 nm ($\Delta\epsilon$ −10.58) and the onset of another negative Cotton effect with maxima below 210 nm ($\Delta\epsilon$ at 210 nm 16.87); NMR (Me_2SO-d_6) δ 9.87 (2 H, br, NH and C=NH^+), 8.52 (2 H, br, NH_2), 7.46 (1 H, d, H-5, $J_{5,3}$ = 1.8 Hz), 6.8 (1 H, d, H-3, $J_{3,5}$ = 1.8 Hz), 6.08 (1 H, s, Hg), 6.35 (1 H, m, H_a, J = 18 and 9.5 Hz), 3.5 (1 H, m, H_b, J = 18 and 9.5 Hz), 2.13–2.5 (2 H, m, H_c and H_d) 2.06 (2 H, br m, H_e and H_f). In TFA the guanidinium protons give three singlets at δ 8.63 (1 H, NH), 7.97 (1 H, C=NH^+) and 7.12 (2 H, NH_2).

Anal. Calcd for $C_{11}H_{12}N_5OBr$·HCl: C, 38.09; H, 3.46; N, 20.20; Br, 23.08; Cl, 10.24. Found: C, 38.23; H, 3.48; N, 19.89; Br, 23.51; Cl, 9.95.

Monobromophakellin (2). Monobromophakellin was obtained by treating the aqueous solution of the hydrochloride with concentrated ammonia. The base was crystallized from methanol to give **2** having mp 260–270 °C (dec): p*K*a 7.6; UV (MeOH) 275 (ϵ 6111) and 228 nm (ϵ 7777); IR (KBr) 3300 (br), 1650, 1620, 1590, 1550, 1480, 1420, 1117, 932, 830, 750 and 618 cm^{-1}; mass spectrum showed M^+ at *m/e* 309 and 311 and fragmentation patterns shown in Scheme I; NMR (Me_2SO-d_6) δ 7.01 (1 H, d, H-5, $J_{5,3}$ = 1.8 Hz), 6.8 (3 H, br, NH and NH_2), 6.6 (1 H, d, H-3, $J_{3,5}$ = 1.8 Hz), 5.52 (1 H, s, Hg), 3.49 (2 H, br t, 13-CH_2), 1.8–2.13 (4 H, br m, 11-CH_2 and 12-CH_2).

Bromination of 2. A solution of monobromophakellin hydrochloride (172 mg) and sodium acetate (82 mg) in glacial acetic acid (5 mL) was reacted at room temperature with 0.5 M bromine solution (1.0 mL) prepared in the same solvent. After stirring for 0.5 h, the solvent was removed under reduced pressure and the residue was dissolved in 2 mL of water. Addition of concentrated ammonia to the solution gave a white precipitate (200 mg) which was identical with 1 in all respects.

Phakellin (3). A solution of diboromophakellin hydrochloride (150 mg) and sodium acetate (100 mg) in methanol was hydrogenated at room temperature and atmospheric pressure over 10% Pd-C catalyst. After the consumption of hydrogen had ceased (1 h), the catalyst was removed by filtration and the filtrate was evaporated under nitrogen to give a white solid. This solid was dissolved in a minimum amount of water and chromatographed over a Rexyn 203 (weak base organic anion exchanger) column in the OH form. Phakellin was eluted by washing the column with water. The water was freeze-dried, and the lyophilized material (57 mg) was crystallized from water to give pure **3**: mp 280 °C (dec); UV (MeOH) 275 and 225 nm; p*K*a 8; IR (KBr), 3440, 1650, 1610, 1590, and 1550 cm^{-1}; mass spectrum M^+ at *m/e* 231.11264 calculated for $C_{11}H_{13}ON_5$ 231.11199; NMR (Me_2SO-d_6) δ 7.01 (1 H, dd, H-5, $J_{5,4}$ = 3, $J_{5,3}$ = 1.8 Hz), 6.56 (1 H, dd, H-3, $J_{3,4}$ = 4 Hz, $J_{3,5}$ = 1.8 Hz), 6.19 (1 H, dd, H-4, $J_{4,3}$ = 4, $J_{4,5}$ = 3 Hz), 5.56 (1 H, s, Hg), 4.1 (3 H, br, NH and NH_2), 3.5 (2 H, br t, 13-CH_2), 1.96 (4 H, m, 11-CH_2 and 12-CH_2). The NMR spectrum of phakellin hydrochloride in Me_2SO-d_6 showed bands at δ 8.62 (2 H, br, NH and C=NH^+), 8.03 (2 H, br, NH_2), 7.38 (H-5), 6.38 (H-4), 6.77 (H-3), 6.12 (1 H, s, Hg), 3.70 (1 H, q, H_a, J = 18 and 8 Hz), 3.51 (1 H, q, H_b, J = 18 and 8 Hz), 2.5–2.15 (2 H, m, H_c and H_d), 2.1 (2 H, br q, H_e and H_f). In F_3AcOH the guanidinium protons showed three singlets at δ 8.65 (1 NH), 8.13 (1, C=NH^+) and 7.18 (2 NH_2).

Monoacetyldibromophakellin (4). The compound was prepared by reacting **1** in pyridine with 1–3 mol of acetic anhydride for 3 h at room temperature. Normal workup followed by crystallization from methanol gave **4**: mp 245 °C (dec); $[\alpha]^{27}_D$ −221 (*c*, 3.15, methylcellosolve); UV (MeOH)[20] 282 (ϵ 8328) and 231 nm (ϵ 18 072); CD (MeOH) 285 ($\Delta\epsilon$ −4.18), 240 ($\Delta\epsilon$ −12.97), and 210 nm ($\Delta\epsilon$ −11.72); IR (KBr) 3360, 3225, 2975, 1710, 1640 (br), 1590 and 1550 cm^{-1}; mass spectrum *m/e* at 429, 431, and 433 M^+; NMR (Me_2SO-d_6) δ 6.86 (1 H, s, H-3), 5.96 (1 H, s, Hg), 4–6 (2 H, br, NH and CH_3CONH), 3.56 (2 H, br, 13-CH_2), 2.02 (7 H, 11-CH_2, 12-CH_2, and CH_3CO). Hydrochloride of **4** in MeOH-d_4 showed bands at δ 7.37 (1 H, s, H-3), 6.47 (1 H, s, Hg), 4.0 (1 H, m, H_a), 3.72 (1 H, m, H_b), 2.6 (2 H, m, H_c and H_d), 2.25 (5 H, H_e, H_f and CH_3CO–).

Anal. Calcd for $C_{13}H_{13}N_5O_2Br_2$: C, 36.13; H, 3.01; N, 16.20; Br, 37.04. Found: C, 36.33; H, 3.09, Br, 37.26.

Transformation of 1 to 10. Dilute nitric acid was prepared by adding 5 mL of water to 2 mL of 70% nitric acid. A solution of 50.0 mg of dibromophakellin in 1.5 mL of dilute nitric acid was heated at 70–75 °C for 5–10 min when evolution of NO_2 started and a white product crystallized out of solution. The white product was collected by suction and crystallized from water to give pure **10** as white needles (25 mg): mp above 300 °C; UV (H_2O) no well defined maxima above 210 nm, ϵ values at 220 and 235 nm are 16 513 and 7339, respectively; IR (KBr) 3250, 1740, 1700, 1648, 1600, and 1564 cm^{-1}; NMR (Me_2SO-d_6) δ 9.42 (br, 2 H, D_2O exchangeable), 8.0 (br, 3 H, D_2O exchangeable), 7.66 (s, 1 H, C(3)–H) 5.80 [s, 1 H, C(6)–H] 3.52–3.09 (two closely spaced multiplets, 2 H, 13-CH_2), 2.1 (br, 2 H, 11- or 12-CH_2) and 1.9 (br, 2 H, 11- or 12-CH_2). In some NMR spectra the five D_2O-exchangeable protons of **10** were found to give four singlets at δ 9.62 [1 H, N(7)–H], 9.3 [1 H, N(9)–H], 8.98 [2 H, C(8)–NH_2], and 7.88 [1 H, OH].

Anal. Calcd for $C_{11}H_{12}N_5O_3Br$·HNO_3H_2O: C, 31.20; H, 3.54; N, 19.90; Br, 18.91. Found: C, 31.23; H, 3.21; N, 20.00; Br, 19.0.

Transformation of 1 to 11. Eight milliliters of water was added to 2 mL of concentrated HCl to prepare dilute hydrochloric acid. Dibromophakellin, 100 mg, was dissolved in 5 mL of dilute HCl. The solution was heated on a boiling water bath until in the UV spectrum of an aliquot the ratio of the intensities of the 237- and 285-nm bands was 3.5 (1 h). Concentration of the reaction mixture under reduced pressure gave a solid which showed four spots on silica gel plates using methanol/acetone/diethylamine (5:5:1) as solvent. Repeated crystallization of this solid from water gave **11** as an amorphous material (16 mg): mp 155–175 °C (dec); R_f 0.21; IR (KBr) 3500–3300 (three bands), 2970, 1700, 1640 and 1590 cm^{-1}; UV (MeOH) 285 (ϵ 3872) and 237 nm (ϵ 16 266); the CD spectrum showed no maxima above 210 nm; NMR (60 MHz) 2.2 (br, 2 H), 2.9 (br q, 2 H), 4.05 (m, 2 H), 7.4 (s, 1H) and 7.9 (br, 4 H, D_2O exchangeable); mass spectrum *m/e* at 387, 389, and 391 (weak, M^+), 370, 372, and 374 (M − NH_3), 345, 347, and 349 (M − NH_2CN), 308 and 310 (M − Br), and 266 and 268 (loss of NH_2CH from M − Br).

Anal. Calcd for $C_{11}H_{11}N_5OBr_2$·HCl: C, 31.02; H, 2.82, N, 16.45; Br, 37.60; Cl, 8.34. Found: C, 30.82; H, 2.56; N, 16.38; Br, 37.52; Cl, 8.12.

Potentiometric Titrations. Analytically pure phakellins (~5 mg) were dissolved in 1.0 mL of carbon dioxide free 40% methylcellosolve–water mixture. The p*K*a values were determined by titrating the magnetically stirred solutions at room temperature with 0.01 N HCl using a Copenhagen pH meter (SDR type made in Denmark) equipped with a glass electrode, a calomel electrode, and a buret capable of delivering 0.025 mL of the titrant accurately. Dibromophakellin gave approximately the same p*K*a value in 80, 60, and 40% methylcellosolve–water mixtures. The p*K*a of **10** was determined in water by titrating with 0.011 N NaOH using a Mettler Automatic Titrator.[19]

X-Ray Crystallography of 4. The precession and Weissenberg photographs showed that the crystals of **4** are orthorhombic and belong to the space group $P2_12_12_1$ as indicated by the systematic absence of reflections *h*00 with *h* odd, 0*k*0 with *k* odd, and 00*l* with *l* odd. A crystal with dimensions 0.26 × 0.35 × 0.30 mm was mounted on a Picker four-circle automatic diffractometer with the *b* axis parallel to the θ axis of the diffractometer. The 2θ values for a number of reflections were measured; the unit cell parameters obtained from least-squares analysis of these measurements are: a = 15.336, b = 12.728, and c = 7.767 Å. The density calculated by assuming that there are four molecules of monoacetyldibromophakellin in the unit cell is 1.872 g cm^{-3}; the density measured by flotation in 1.885 g cm^{-3}.

The three-dimensional intensity data were collected with nickel-filtered Cu radiation using a $\theta - 2\theta$ scanning technique. Measurements were made for 1339 independent reflections in the range $4° \leq 2\theta \leq 100°$. Two standard reflections were measured after every 50 reflections to check the stability of the instrument and the stability of the crystal. The fluctuations in the intensity of standard reflections were less than 1%. Background counts of 20 s were taken at each end of the scan. The intensities were corrected for Lorenz and polarization factors and were placed on an approximate absolute scale by a constant obtained from a Wilson plot. All reflections were used including unobservable ones.[16] Corrections for absorption effects were applied because the magnitude of the linear absorption coefficient (μ = 68.18 cm^{-1}) was high.

A three-dimensional $E^2 - 1$ Patterson synthesis was computed to locate the two bromine atoms. The map showed seven peaks of sufficient densities to be Br–Br vectors. The peaks at 0.0 and ½ (intramolecular Br–Br vectors) and 0.276, 0.375, and 0.0 [vectors between Br(4) and Br(5) of molecules related by screw axis operations along *z*] suggested that the two heavy atoms have the same *x*, *y* coordinates and that these atoms are separated by ½ along *z* axis. This interpretation was consistent with the presence of remaining Br–Br vector peaks in the Patterson map.

The *x* and *y* coordinates of Br(4) and Br(5) were deduced from the

peak located at $w = 1/2$. The four peaks in the planes $\mu = 1/2$ and $v = 1/2$ gave two values for the z coordinates of Br(4). A choice between these values could not be made at this stage of the analysis because it was not possible to decide which two of the four peaks represent vectors between symmetry-related bromine atoms. The second bromine atom, Br(5), was assigned the z coordinate of $1/2$ + the z value of Br(4). The two sets of coordinates thus obtained for Br(4) and Br(5) are listed as follows; set I: Br(4) 0.1160, 0.1875, −0.1625; Br(5) 0.1160, 0.1875, 0.3375; set II: Br(4) 0.1160, 0.1875, −0.090; Br(5) 0.1160, 0.1875, 0.410.

Although calculation of structure factors with the atomic positions listed in sets I and II gave the same value (0.50) for the residual index ($R = \sum \|F_0| - |F_c\| / \sum |F_0|$), the electron-density maps computed from these sets of coordinates were quite different. Only the electron-density map derived from the atomic coordinates listed in set I showed features of a chemically meaningful structure. In this first three-dimensional Fourier map, the six-membered ring B, the five-membered ring D, and the four atoms of the pyrrole ring A of structure **4** were clearly discernible. Eight of the best-defined atoms were chosen as the basis of second Fourier in which all 22 nonhydrogen atoms belonging to **4** appeared distinctly. A third Fourier based on all 22 atoms gave a structure in which all atoms were well defined and no false peaks were present. The atomic coordinates were now refined and used as a basis for fourth Fourier which gave $R = 0.30$. The R was reduced to 0.13 through least-squares refinement on isotropic thermal parameters and coordinates. In these refinements the nitrogens were treated as carbons because the identity of these atoms could not be inferred from the electron-density maps. The oxygen and bromine atoms were clearly distinguishable from F_0 synthesis.

The nitrogen atoms were identified as follows: All atoms, except oxygens and bromines, were assigned an atomic scattering factor of carbon and temperature factor of $B = 3.0$ Å.[2] Least-squares refinement on temperature factors showed five atoms had a value of $B \simeq 1/2$ of that of other atoms (Table IV, Supplementary Material). This suggests that the number of electrons assigned to these atoms are insufficient. Consequently, these five atoms were considered to be nitrogens. A second least-squares refinement on isotropic temperature factors and positional parameters, using the correct structure factors for C, N, O, and Br atoms, gave reasonable values of B for all atoms (Table IV) and a residual index of 0.09, changing isotropic to anisotropic temperature factors lowered the residual index to 0.056.

A difference electron-density map revealed the positions of 11 of the 13 hydrogen atoms. The H(11B) and H(12B) were not observed. Placement of 11 hydrogens and inclusion of anomalous scattering factor contributions for two bromine atoms followed by still further least-squares refinements lowered the reliability index to the current value of 0.040 for the structure. The residual index for the mirror image was 0.048. This statistically significant difference suggests[17] that the structure and not its mirror image is the correct absolute configuration. The structure of monoacetyldibromophakellin as presented in Figure 1 was now considered to be correct, and it may well be presumed that the parent alkaloid, dibromophakellin, has structure **1**.

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Supplementary Material Available. A complete listing of coordinates and structure factor amplitudes of all atoms (Table I) and the bond distances and bond angles (Table II). The least-squares planes and deviation of individual atoms from these planes (Table III) and the temperature factor refinement for the identification of nitrogen atoms (Table IV). Ordering information is given on any current masthead page.

Registry No.—**1**, 31954-96-8; **1** HCl, 63700-27-6; **2**, 31955-05-2; **2** HCl, 63700-28-7; **3**, 33051-47-7; **3** HCl, 31955-03-0; **4**, 31955-04-1; **4** HCl, 63700-29-8; **10**, 63626-31-3; **11**, 63626-32-4.

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(8) Dibromophakellin did not furnish crystals suitable for x-ray analysis. Treatment of monoacetyldibromophakellin **4** with 70% methanol at room temperature for 2 days gave a product which was confirmed to be dibromophakellin **1** by IR, UV, CD, NMR, and mass spectral data. The results of this experiment suggest that in **4** the main skeleton of the parent compound remains unaltered.
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(16) Using the criterion $I \leq 1.5\ \sigma(I)$, 48 of the 1339 reflections were considered to be unobservable.
(17) W. S. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965); W. C. Hamilton, "Statistics in Physical Science", Ronald Press, New York, N.Y., 1964.
(18) We are grateful to Dr. F. M. Lovell of Lederle Laboratories, Pearl River, N.Y. 10964, for communicating to us the results of x-ray analysis of **10** prior to publication.
(19) We express our gratitude to Mr. G. P. McTernan of Lederle Laboratories, Pearl River, N.Y. 10965, for measuring the pKa value of **10**. Mr. McTernan also determined the pKa values of **2** and **3**, using a Model E-436 Metrohm Potentiograph. His results and ours were in agreement within experimental errors.
(20) The increase in the intensity of the short wavelength band upon going from **1** to **4** is due to the superposition of the absorbtions by the pyrrole ring and the acetylaminoimidazoline ring; see ref 11.
(21) D. John Faulkner and Raymond J. Andersen. "The Sea", Vol 5, Wiley, New York, London, Sydney, and Toronto, 1974, p 679–714.
(22) Another factor which may reduce the basicity of the guanidino group of phakellins would be the negative inductive ($-I$) effects of the pyrrole and amide nitrogen atoms attached to C(6) and C(10) of the imidazoline ring.