result that the first and second π ionization energies of $PhBF_2$ are greater than those of $PhBCl_2$. There is, therefore, a reversal of the substituent effect order based on NMR chemical shifts. The postulate of a dominant BF_2 field effect also offers a rationale for the observation that although according to the CNDO/2 calculation the BF_2 group withdraws less π-electron density from the benzene ring than even BR_2, the ^{13}C NMR derived σ^0_R and σ^+_p values of BF_2 are more positive than those of BR_2.

Still greater p–π back-bonding to boron by oxygen in $PhB(OH)_2$ and $PhB(OCH_3)_2$ reduces π-electron withdrawal from benzene by BX_2 to the point that the electron-donating inductive effect in the sigma framework finds expression in the negative σ_I values, Table II. The small calculated π-electron withdrawal of 0.05 electron from the ring by $B(OH)_2$ is however sufficient to account for the observed para ^{13}C NMR chemical shifts when a shift factor of the order of 2×10^2 ppm/electron[7] is assumed.

It is interesting to compare the ^{13}C NMR chemical shifts of the monoarylboranes $PhBX_2$ with recently reported[11] NMR data for triphenylborane (Table I) and with trimesitylborane ^{13}C NMR shifts relative to mesitylene, which we have also measured.

The small δ ^{13}C para and negative δ ^{13}C meta NMR shifts relative to benzene reported[10] for Ph_3B seem in keeping with the known twisted propeller conformation of the triarylboranes which reduces 2p–π interactions between boron and the benzene ring. Comparable ^{13}C chemical shifts were measured for trimesitylboron relative to mesitylene (δ para 2.5 δ meta 1.7). The ortho carbons of Ph_3B, however, show an exceptionally large downfield shift of almost 10 ppm, which is even more surprising in view of the small ortho ^{13}C shift of trimesitylborane, relative to mesitylene, δ ^{13}C ortho 2.9, when compared to the other ortho ^{13}C NMR shifts of the $PhBX_2$ series. (The ortho and para methyl carbons of trimesitylborane exhibit downfield shifts of 2.0 and 0.1 ppm, respectively, relative to mesitylene.) It would appear that ortho carbon ^{13}C chemical shifts of triarylboranes may be sensitive functions of the aryl group twist angle relative to the plane of the boron–carbon bonds. This propeller twist angle is greater in trimesitylborane, because of the steric bulk of the ortho methyl groups, than in triphenylborane.[12] At small twist angles, ortho positions of triarylboranes will be deshielded by adjacent aromatic rings,[12] as well as by the deshielding anisotropy which seems to be associated with B–sp^2 bonds in the $PhBX_2$ sereis. At twist angles approaching 45°, the ortho ring positions may move into shielding regions of both B–sp^2 bonds, analogous to those of C=O or NO_2, and of the adjacent aromatic rings.[13]

If magnetic anisotropy, associated with adjacent rings and the central boron atom, does play a significant role in the ^{13}C NMR shifts of triarylboranes, the role of similar effects will have to be reevaluated in the spectra of the isoelectronic and isosteric triarylcarboniums, where they have been neglected[6,13] in favor of charge density effects. There is, in general, a parallel between NMR shifts observed for carbonium ions and corresponding boranes, for example, $PhCF_2^+$,[13,14] $PhCMe_2^+$,[15,16] and $PhC(OMe)_2^+$.[16,17]

Following submission of this manuscript, an extensive study appeared[17] of the ^{13}C spectra of phenylboranes and boron-substituted aromatic heterocycles. Between this most recent paper and our own there is no disagreement as to experimental details; in the interpretation of the results we differ most in drawing attention to the strong inductive-field effects of BCl_2, BF_2, and $B(OR)_2$ groups.

Experimental Section

Details of the synthesis of $PhBX_2$ (X is F, OCH_3) and of the modified CNDO/2 calculations (X is Cl, F, OH, and CH_3) are provided in ref 2.

All NMR spectra were run in either the CW (for ^1H NMR) or FT (^{13}C NMR) mode on a Varian XL-100 spectrometer with internal D lock; ^1H (100 MHz), ^{13}C (25.2 MHz).

Both ^1H and ^{13}C NMR spectra of $PhBX_2$ (X is F, Cl, OCH_3, and 9-BBN) were run on 15% v/v solutions in $DCCl_3$ with internal cyclohexane as a reference. Conversions to chemical shifts relative to benzene were made with the following shift conversions: δ^{CH}_{PhH} ^1H 5.94 ppm; δ^{CH}_{PhH} ^{13}C 101.16 ppm; in $DCCl_3$, ref 8a.

Spectra of $PhB(OH)_2$ were run in CD_3OD (saturated solution) referenced to internal tetramethylsilane and converted to benzene reference by $\delta^{Me_4Si}_{PhH}$ ^1H 7.37 ppm; $\delta^{Me_4Si}_{PhH}$ ^{13}C 128.50 ppm, ref 8a.

Spectra of trimesitylborane were run in C_6D_6 referenced to internal tetramethylsilane. Chemical shifts of mesitylene, $\delta^{13}C$, were taken from ref 6, corrected to $\delta^{Me_4Si}_{PhH}$ ^{13}C 128.6 ppm. Chemical shifts, $\delta^{13}C$, relative to tetramethylsilane, of trimesitylborane were 21.23 (*p*-CH_3), 23.07 (*o*-CH_3), 129.02 (*m*-C), 140.25 (*p*-C), and 140.57 (*o*-C) ppm.

Acknowledgment. We acknowledge the support of a Lubrizol Corporation Grant of the Research Corporation.

Registry No. $PhBCl_2$, 873-51-8; $PhBF_2$, 368-98-9; $PhBR_2$, 72826-88-1; $PhB(OH)_2$, 98-80-6; $PhB(OMe)_2$, 13471-35-7.

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Isolation and Structure of Bohemamine, (1aβ,2α,6aβ,6bβ)-3-Methyl-*N*-(1a,6,6a,6b-tetrahydro-2,6a-dimethyl-6-oxo-2*H*-oxireno[*a*]pyrrolizin-4-yl)-2-butenamide

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While screening cultures of actinomycetes for novel antitumor substances we have isolated an anthracycline mixture, bohemic acid complex, from *Actinosporangium* sp. strain C36145 (ATCC 31127).[1] In addition to the known antibiotics cinerubins A and B[2] and pyrromycin,[3]

(1) It is the practice of this laboratory (B.L.) to give trivial names to antibiotic complexes based on operas. This complex is named for La Bohème and compounds subsequently isolated from the complex are given trivial names based on characters in the opera.
(2) W. Keller-Schierlein and W. Richle, *Antimicrob. Agents Chemother.*, **1970**, 68 (1971).

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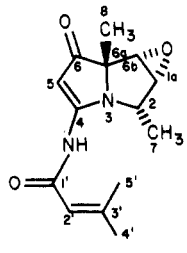
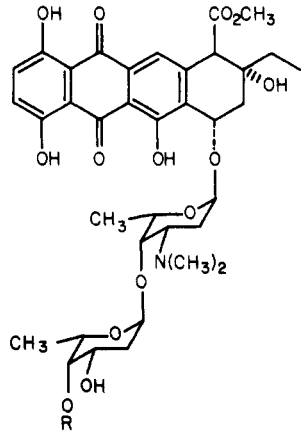
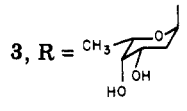


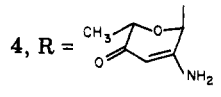
1, bohemamine



2, R = H musettamycin



3, R = marcellomycin



4, R = rudolphomycin

Figure 1. Structures of bohemic acid culture products.

fractionation of the complex has yielded six new anthracycline antitumor agents, among them musettamycin (2), musettamycin (3), and rudolphomycin (4) (Figure 1), which have been the subjects of earlier communications from our laboratory.[4-6] Fractionation of the complex into its basic and neutral components greatly simplified the mixture and yielded the nine previously mentioned anthracycline aminoglycosides, traces of other anthracycline components, and a relatively major nonanthracycline component, 1. In this note we report the isolation and structure determination of compound 1, which we have named bohemamine.

Initially the isolation of 1 was accomplished via chromatography of a mixture of 1, 2, and 4 on Sephadex LH-20 by using chloroform as an eluant.[5] This gave 1 as a crystalline solid having a reddish orange color due to contamination with anthracyclines. Subsequently it was found that 1 could be prepared free of 2 and 4 by the simple expedient of washing a methylene chloride solution of 1, 2, and 4 with aqueous copper sulfate (0.1 M). This caused the majority of the anthracyclines to precipitate as their copper complexes and the remainder to partition to the aqueous phase. 1 was obtained from the organic phase as a pale-pink solid.

Bohemamine (1) could be recrystallized from methylene chloride–ether as white crystals, mp 199–200 °C dec. It exhibited IR bands characteristic of an α,β-unsaturated secondary amide and a 5-membered α,β-unsaturated ketone (IR bands at 3280, 3190, 1710, 1635, 1560, 1510 cm^{-1}). The UV spectrum of 1 in methanol showed three maxima at 248, 286, and 335 nm. On addition of dilute acid, the bands at 286 and 335 nm shifted to 268 and 331 nm, respectively, consistent with the expected behavior of a β-amino-α,β-unsaturated ketone.[7] The elemental formula, $C_{14}H_{18}N_2O_3$, was determined by elemental analysis and high-resolution mass spectrometry. In addition to the molecular ion at m/e 262, ions at 179 ($C_9H_{11}N_2O_2$), 163 ($C_9H_{11}N_2O$), 151 ($C_8H_9NO_2$), 112 ($C_6H_{10}NO$), 96 ($C_6H_{10}N$), 83 (C_5H_7O), 68 (C_3H_2NO), and 55 (C_4H_7) were observed with the ion at m/e 83 being the parent ion. The ^1H NMR spectrum of 1 in $CDCl_3$ indicated the presence of methyl groups at δ 1.38 (s, 3 H) and 1.57 (d, 3 H, J = 7 Hz), vinylic methyl groups at δ 1.94 (d, 3 H, $J \simeq 1$ Hz) and 2.28 (d, 3 H, $J \simeq 1$ Hz), a narrow AB quartet at δ 3.58 (AB quartet, 2 H), a quartet at δ 3.87 (q, 1 H, J = 7 Hz), vinyl proton signals at δ 5.67 (s, 1 H) and 5.88 (br s, 1 H), and an exchangeable proton at δ 9.2 (br s, 1 H). It is notable, in retrospect, that the signals at δ 3.58 and 3.87 exhibited no further coupling. The ^{13}C NMR spectrum (Table I) showed two carbonyls at δ 204.4 and 169.6, assignable to ketone and amide, respectively.

From the above data a number of partial structures could be deduced. The presence of the $(CH_3)C{=}CH{-}CO$ fragment was shown by the ^1H and ^{13}C NMR spectra, the ion in the mass spectrum at m/e 83, and the IR carbonyl band at 1635 cm^{-1}. The failure to observe an ion in the mass spectrum at m/e 99 for $C_5H_7O_2$ coupled with the presence of an amide NH suggested the further partial structure $(CH_3)_2C{=}CHCONH$. The signals at δ 92.4, 164.1, and 204.4 in the ^{13}C NMR of 1 were similar to those seen for the three sp^2 carbons in the terminal sugar residue of rudolphomycin (4), which suggested the structural fragment $N{-}C{=}CH{-}CO$.[8] The presence of the vinylic proton at δ 5.67 in the ^1H NMR spectrum supported the assignment, as did the UV bands at 286 and 335 nm. The above fragments accounted for all but 6 of the original 14 carbon atoms in the compound. The signals in the ^{13}C NMR at δ 73.6, 63.9, 56.3, and 55.8 together with the two methyl carbons and the ^1H NMR suggested two further fragments, $CH_3{-}C{-}X{-}CHCH_3$ and $CH(X){-}CH(X)$. The possibility that the latter fragment could be cyclized to an aziridino or epoxido fragment was suggested by the unsaturation of the molecule which necessitated at least three rings. In view of the lack of coupling of the epoxido–aziridino protons to the remaining methine, no reasonable structure could be drawn and a single-crystal X-ray structure determination was carried out.

Figure 2 shows a computer-generated perspective drawing of the final X-ray model less hydrogens. The X-ray experiment defined only the relative configuration, so the enantiomer shown represents an arbitrary choice. The bridgehead nitrogen atom is midway between sp^2 and sp^3 hybridization, and this presumably accounts for the weakly basic nature of bohemamine. The vinylogous amide ring is planar within experimental error, and the two five-membered rings have an interplanar angle of 52° with the epoxide on the concave face. The 3-methyl-2-buten-

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(8) The observed similarities in the spectra of 1 and 4 motivated our work on the structure determination of 1.

Table I. ^{13}C NMR Spectrum of 1[a]

C-7	14.3 q	C-5	92.4 d
C-2	63.9 d	C-4	164.1
C-1A	(55.8) d	C-1′	169.6
C-6B	(56.3) d	C-2′	117.5 d
C-6A	73.6	C-3′	158.1
C-8	19.1 q	C-4′	20.5 q
C-6	204.4	C-5′	27.8 q

[a] Run at 25 MHz in $CDCl_3$. Unmarked signals are singlets. Signals in parentheses may be interchanged.

amide fragment is essentially at right angles to the vinylogous amide ring. The dihedral angle between H(1a) and H(2) is 45°, a surprising value in view of the lack of observed coupling between these protons in the ^1H NMR. In general, bond distances and angles agree well with expected values.

Bohemamine has an unusual tricyclic structure somewhat reminiscent of the pyrrolizidine alkaloids, but the substitution pattern is not consistent with known members of this family or the proposed biosynthesis of these materials.[9] Recently the isolation of the antibiotics clazamycin A and B, **5** and **6**, and the possibly related 2-hydroxy-5-iminoazacyclopent-3-ene (**7**) has been reported.[10] The structures of **5** and **6** (Figure 3) are quite similar to that of bohemamine (**1**). Conceivably **1**, **5**, and **6** have similar biosynthetic origins. Bohemanine (**1**) has been tested for antibiotic, antifungal, and antitumor activity and has been found to be inactive.

Experimental Section

Isolation of Bohemamine (1). To a solution of 5.84 g of a mixture of **1**, **2**, and **4** (obtained via preparative LC/500 chromatography of bohemic acid complex[5]) in 200 mL of CH_2Cl_2 was added 200 mL of 0.1 M aqueous $CuSO_4$. The mixture was shaken and an orange-brown precipitate resulted. The emulsion was centrifuged and the layers separated. The aqueous layer was washed with CH_2Cl_2 (2 × 200 mL), adjusted to pH 7.5 with 0.1 M $NaHCO_3$, and extracted with an additional 300 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated to yield 1.004 g of an orange solid greatly enriched in **1**. The solid was taken up in 100 mL of CH_2Cl_2 and washed with 0.1 M $NaHCO_3$ (100 mL). The organic layer was dried over Na_2SO_4 and evaporated to yield 0.442 g of **1** contaminated by anthracyclines. The aqueous layer was saturated with NaCl and extracted with CH_2Cl_2 (4 × 100 mL). The extracts were evaporated to yield 0.383 g of **1** as a pale-pink solid. Recrystallization of **1** from CH_2Cl_2–ether gave 187 mg of **1**: mp 199–200° C dec; IR (KBr) 3280, 3190, 1710, 1635, 1560, 1510 cm^{-1}; UV λ_{max} (MeOH) 248, 286, 335 nm; UV (MeOH–HCl) 248, 268, 331 nm.

Anal. Calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68; mol wt, 262.3114. Found: C, 63.78, 64.05; H, 7.00, 7.08; N, 10.44, 10.54; mol wt (mass spectroscopy), 262.3152.

Other significant peaks in the high-resolution mass spectrum appeared at *m/e* (composition, %) 179 ($C_9H_{11}N_2O_2$, 2.66), 163 ($C_9H_{11}N_2O$, 5.84), 151 ($C_8H_9NO_2$, 4.53), 112 ($C_6H_{10}NO$, 12.02), 96 ($C_6H_{10}N$, 6.72), 83 (C_5H_7O, 100), 68 (C_3H_2NO, 14.84), 55 (C_4H_7, 47.88). ^1H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 1.57 (d, 3 H, J = 7.0 Hz), 1.94 (d, 3 H, $J \simeq 1$ Hz), 2.28 (d, 3 H, $J \simeq 1$ Hz), 3.58 (AB quartet, 2 H), 3.87 (q, 1 H, J = 7.0 Hz), 5.67 (s, 1 H), 5.88 (br s, 1 H), 9.2 (br s, 1 H, exchanged with D_2O).

Crystals of bohemamine (**1**) were grown by slow evaporation of MeOH–diisopropyl ether solutions. Preliminary X-ray photographs revealed orthorhombic symmetry, and accurate lattice constants were determined from a least-squares fitting of 15 moderate 2θ values measured on a previously aligned diffractometer. The cell dimensions were a = 8.50 (1), b = 8.564 (1), and c = 19.20 (1) Å. A rough density measurement (1.24 g/cm^3), the known chirality, and the systematic extinctions uniquely indicated space group $P2_12_12_1$, with one molecule of $C_{14}H_{18}N_2O_3$ per asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a fully automated four-circle diffractometer using Cu Kα radiation (1.541 78 Å) and a 1° ω scan. A total of 1163 unique reflections was surveyed in this manner, and 1127 (97%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects.[11] The angular dependence of the scattering was removed as the intensity data were converted to normalized structure factors.[11] An initial phasing model was achieved by using a multisolution weighted tangent formula approach.[11] This scheme revealed all nonhydrogen atoms and hydrogens were located on a subsequent difference Fourier synthesis. Full-matrix, least-squares refinements have lowered the conventional crystallographic residual to its current minimum of 0.071 for the observed reflections.

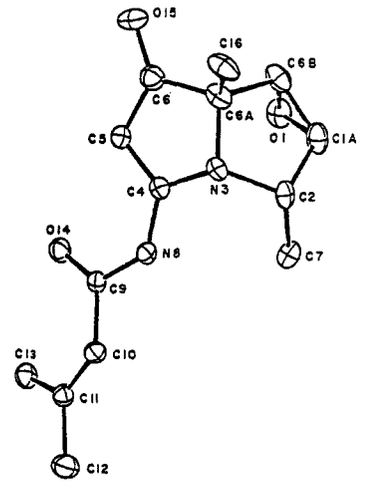

Figure 2. Computer-generated perspective drawing of 1 less hydrogens.

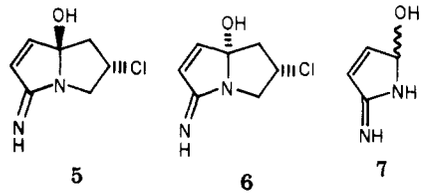

Figure 3. Structures of clazamycin A (**5**), clazamycin B (**6**), and 2-hydroxy-5-imino-1-azacyclopent-3-ene (**7**).

Acknowledgment. We greatly acknowledge the partial support of this work under Contract NO1–CM–77147 from the Division of Cancer Treatment, National Institutes of Health, Education, and Welfare. We also thank Dr. K. Loening of Chemical Abstracts Service for help in naming bohemamine. This work was partially supported by an NIH–NCI grant (J.C.) and the diffractometer used was funded by NSF.

Registry No. 1, 72926-12-6.

Supplementary Material Available: Tables II–IV listing final atomic and anisotropic thermal parameters, bond distances, bond angles, and their errors (3 pages). Ordering information is given on any current masthead page.

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