monocobalt compounds (e.g., **3**), which have two or more voltammetric waves, but only one (the reduction to the monoanion) is reversible in acetonitrile. In **3**, for example, reduction of the monoanion occurs in a one-electron step at −2.50 V, but the resulting dianion is extremely unstable and the wave is irreversible even at rather high (10 V/s) scan rates.

The greatly enhanced stability of the dicobalt dianions is most likely due to the ability of the cluster to spread the negative charge over both metals. This kinetic stability could occur if the anions have a delocalized electronic structure, or if there is charge isolation on the cobalts (e.g., two formally Co(II)'s in the dianions). Our electrochemical results strongly favor the delocalized electronic structure, for if there were strictly isolated cobalt reduction sites, or very weak electronic coupling, we would expect to find either a single two-electron wave, or two closely spaced one-electron waves.[10] Although the electrochemical data cannot offer a quantitative description of the lowest unoccupied molecular orbital (LUMO) of the triple deckers, it seems clear that there is significant charge delocalization in the anions. This is consistent with the results of a molecular orbital treatment of this class of compounds by Lauher and co-workers,[11] in which it was shown that there is significant mixing of the metal and central ligand σ orbitals, and that the LUMO for **1** is weakly *bonding* between the metals.

The stable ions generated in our electrochemical experiments contain from 29 to 32 valence electrons ($\mathbf{1}^{+}$ to $\mathbf{1}^{2-}$), if the usual conventions for electron counting are followed. The LUMO orbital for neutral **1** (30 electrons) is actually a degenerate (e_1') set which is fully occupied in the 34-electron $Cp_3Ni_2^{+}$. This orbital is high in metal character and nonbonding with respect to the central ring.[11] Since the highest occupied orbital (HOMO) of **1** is also a nonbonding metal orbital,[11] it is not surprising that the several redox steps do not seem to affect the metal–ligand bonding in any catastrophic way. It does not seem that the degree of occupancy of the e_1' set is critical to the stability of triple deckers. We note in this regard that Siebert and co-workers have recently isolated neutral triple deckers with various metals, containing 30 to 33 electrons.[12]

The above discussion employed a model in which the triple deckers were viewed as delocalized, multi-metal, π-complexes. A popular alternative model would view **1–4** as cluster compounds derived from the pentagonal-bipyramidal structure of $C_2B_5H_7$ by replacement of one or two BH vertices by $CpCo^{2+}$. The similarity of $CpCo^{2+}$ and BH bonding in molecular clusters has been pointed out previously,[13-15] and the neutral cobalt clusters retain the closo shape with $2n + 2$ framework electrons.[16] Taken in this context, the apparent absence of major structural changes in going to the $Cp_2Co_2C_2B_3H_5^{2-}$ dianions might be surprising because electron-counting rules predict a nido structure for a $2n + 4$ electron species.[16] Comparing these seven-vertex dicobalt clusters with smaller clusters, we note that when the closo tetrahedral cluster $Co_3(CO)_9CR$ is reduced,[17] an extremely unstable dianion is produced, and, similarly, reduction beyond the monoanion stage has proved fatal for other metal clusters as well.[18,19]

Since Hawthorne and co-workers have reported extensively on the electrochemical properties of large metallocarboranes,[20] clusters of this general type may provide a model system to investigate the effects of size and stoichiometry on the redox properties of molecular clusters. We will comment on these aspects in more detail in a subsequent paper.

Acknowledgments. The authors are indebted to R. N. Grimes for his generosity in providing samples of **1–4.** The support of the National Science Foundation (CHE 76-83668) is also gratefully acknowledged.

References and Notes

(1) Werner, H., Salzer, A. *Synth. Inorg. Met.-Org. Chem.* **1972,** *2,* 239. Salzer, A., Werner, H. *Angew. Chem., Int. Ed. Engl.* **1972,** *11,* 930.
(2) Werner, H., *Angew. Chem., Int. Ed. Engl.,* **1977,** *16,* 1.
(3) Robinson, W. T.; Grimes, R. N., *Inorg. Chem.* **1975,** *14,* 3056.
(4) Beer, D. C.; Miller, V. R.; Sneddon, L. G.; Grimes, R. N.; Mathew, M.; Palenik, G. J. *J. Am. Chem. Soc.* **1973,** *95,* 3046.
(5) Grimes, R. N.; Beer, D. C.; Sneddon, L. G.; Miller, V. R.; Weiss, R. *Inorg. Chem.,* **1974,** *13,* 1138.
(6) Miller, V. R.; Grimes, R. N. *J. Am. Chem. Soc.,* **1973,** *95,* 2830.
(7) Most experiments were performed in either acetonitrile or dichloromethane. The supporting electrolyte was tetrabutylammonium hexafluorophosphate (0.1 M), and potentials are referenced to the aqueous saturated calomel electrode. Since these compounds are all air-stable, nitrogen purging was sufficient to exclude air from electrochemical cells. Experimental procedures are generally as given in: Bowden, W. L.; Holloway, J. D. L.; Geiger, W. E. *Inorg. Chem.* **1978,** *17,* 256.
(8) Meites, L. "Polarographic Techniques", 2nd ed; Interscience: New York, 1965.
(9) Nicholson, R. S.; Shain, I. *Anal. Chem.,* **1964,** *36,* 706.
(10) For a discussion of these ideas, readers are referred to several recent papers: Morrison, W. H.; Krogsrud, S.; Hendrickson, D. N. *Inorg. Chem.* **1973,** *12,* 1998; Morrison, W. H.; Ho, E. Y.; Hendrickson, D. N. *Ibid.* **1975,** *14,* 500; Fenton, D. E.; Schroeder, R. R.; Lintvedt, R. L. *J. Am. Chem. Soc.,* **1978,** *100,* 1931; Flanagan, J. B.; Margel, S; Bard, A. J.; Anson, F. C. *Ibid.* **1978,** *100,* 4248.
(11) Lauher, J. W.; Elian, M.; Summerville, R. H.; Hoffmann, R. *J. Am. Chem. Soc.* **1976,** *98,* 3219.
(12) Siebert, W.; Edwin, J.; Bochmann, M. *Angew. Chem., Int. Ed. Engl.* **1978,** *17,* 868.
(13) Elian, M; Hoffmann, R. *Inorg. Chem.,* **1975,** *14,* 1058.
(14) Mingos, D. M. P.; Forsyth, I. M. *J. Chem. Soc., Dalton Trans.* **1977,** 610.
(15) Mingos, D. M. P. *J. Chem. Soc., Dalton Trans.* **1977,** 602.
(16) Wade, K. *Adv. Inorg. Chem. Radiochem.* **1976,** *18,* 1.
(17) Bond, A. M., Peake, B. M.; Robinson, B. H.; Simpson, D.; Watson, D. J. *Inorg. Chem.* **1977,** *16,* 410.
(18) Bond, A. M.; Davison, P. A.; Peake, B. M.; Robinson, B. H.; Simpson D. *Inorg. Chem.* **1977,** *16,* 2199.
(19) Mikolova, D.; Klima, J.; Vlcek, A. A. *Inorg. Chim. Acta* **1978,** *27,* L67.
(20) Cyclic voltammetry results have been reported by Hawthorne and coworkers for most of the metallocarboranes they have synthesized. Leading references can be found in reviews of metallocarborane chemistry. E.g., Hawthorne, M. F. *Pure Appl. Chem.* **1973** *33,* 475. Callahan, K. P.; Hawthorne, M. F. *Pure Appl. Chem.* **1974** *39,* 475. Grimes, R. N. "Organometallic Reactions and Syntheses", Vol. 6, Becker, E. I.; Tsutsui, M. Eds.; Plenum: New York, 1977.

David E. Brennan, William E. Geiger, Jr.*
Department of Chemistry, University of Vermont
Burlington, Vermont 05401
Received December 4, 1978

Mapping of Cocaine and Cinnamoylcocaine in Whole Coca Plant Tissues by MIKES

Sir:

Methodology is presented which allows the distribution of organic compounds in plant tissue to be mapped accurately on a dimensional scale as small as 1 mm^3. Individual tissue specimens are examined so that averaging over a population is avoided; no sample preparation or prefractionation is needed, and total analysis time is only a few minutes per sample. In addition to demonstrating this organic mapping capability, we show that different parts of a plant as well as plants from different regions can be differentiated on the basis of alkaloid content. The data presented are for *Erythroxylum coca* Lam., and they have immediate analytical implications for chemotaxonomy and plant physiology; however, the approach used is likely to be more generally applicable.

Analysis is by MIKES (mass-analyzed ion kinetic energy spectrometry), i.e., by sequential two-stage mass spectrometry with dissociation of selected ions in the interanalyzer region and mass analysis of the resulting fragments in the second analyzer. The direct (nonchromatographic) analysis of individual trace organic constituents of complex mixtures is thus possible.[1-4] The present experiments employ direct probe vaporization, chemical ionization, and collision-induced dissociation. Cocaine (benzoylmethylecgonine) and cinnamoylco-

0002-7863/79/1501-3400$01.00/0 © 1979 American Chemical Society

Table I. Distribution of Cocaine and Cinnamoylcocaine in *Erythroxylum coca* Lam.

ascessions	plant part	% cinnamoylcocaine	(cocaine)/(cinnamoylcocaine)
Tingo Maria	leaf, powdered (control)	2.3[a]	43[a]
Tingo Maria	leaf, margin, A, B, H, I, K[b]	4.2, 3.6, 2.3, 1.8, 1.1	23, 27, 42, 54, 90
Tingo Maria	leaf, center (with veins), A	6.1	15
Tingo Maria	leaf, center (no veins), G, K	2.6, 1.1	37, 90
Tingo Maria	leaf, stem, A	6.4	15
Tingo Maria	twig, C, D. E	33, 30, 9	2.1, 2.3, 10
Tingo Maria	berry, powdered (control)	51	1.0
Tingo Maria	berry, center (white)	*d*	*d*
Tingo Maria	berry, inside (brown)	68	0.47
Tingo Maria	berry, outside (black)	33	2.0
Tingo Maria	berry, stem	50	1.0
Trujillo[c]	leaf, powder (control)	9.0	10
Trujillo[c]	leaf, margin, F	8.5	11
Trujillo[c]	parasitic material (blank)	*d*	*d*
Trujillo[c]	berry, center (white)	*d*	*d*
Trujillo[c]	berry, stem	20	4.0

[a] As an example of the precision of the method, the standard deviation ($n = 6$) of this determination is 0.3 corresponding to an alkaloid ratio of 43 ± 6. The calculated percent cinnamoylcocaine assumes equal ion yields for the two alkaloids which is true within the error of the experiment. [b] Results are for arbitrarily selected leaves; letters designate particular samples. [c] Results are for one set of data only. [d] Alkaloid content too low for measurement.

caine (cinnamoylmethylecgonine) were monitored using a single reaction monitoring technique in which each alkaloid was characterized by its molecular weight and one dissociation (loss from the protonated molecules of benzoic acid and cinnamic acid, respectively).[5] This was accomplished at constant magnetic field by simultaneously altering the ion-accelerating voltage and the electric sector voltage. This procedure,[6] as well as other aspects of MIKES germane to these experiments, has been fully reported.[7-9]

Plant material[10] consisted of two collections (designated Tingo Maria and Trujillo representing different geographic regions in Peru) of *Erythroxylum coca* Lam. In each case, leaves, stems, and berries were available. Leaves of different sizes and ages (1-g total) were pulverized, powdered, and homogenized to serve as controls. Portions of each sample have been retained as reference materials.

The relative concentrations of cocaine and cinnamoylcocaine were estimated in various portions of the Tingo Maria collection by monitoring characteristic reactions of each protonated alkaloid ($304^+ \rightarrow 182^+$ and $330^+ \rightarrow 182^+$, respectively) as material was vaporized from the sample.[11] Total peak areas (Figure 1) were used in quantitating the relative abundance of the two alkaloids so that the results would be independent of matrix effects on vaporization. In fact, cocaine showed an optimum vaporization temperature of 185 °C which was independent of the section of the plant material examined. Cinnamoylcocaine exhibited a variable optimum vaporization temperature of 195–230 °C depending on the section of the plant material investigated. These differences in the vaporization profiles for the two alkaloids do not occur for leaf margins of either collection, and they indicate that the profiles themselves may be a source of information on alkaloid site and/or binding in the plant.

Table I summarizes the results for both plant accessions covering various parts of each plant. Whole powdered leaves and berries were examined as a control before and after each set of determinations. The results showed excellent reproducibility for repeated runs of split samples (Table I). The main findings from the Tingo Maria data are (i) the leaves contain the highest relative concentration of cocaine, (ii) individual leaves show wide variations (factor of 4) in cocaine/cinnamoylcocaine ratio even when the same leaf parts are sampled, (iii) leaf margins exhibit lower cinnamoylcocaine concentrations (relative to cocaine) than do the centers unless veins are removed, (iv) leaf stems (and veins) contain relatively more cinnamoylcocaine than the leaf margins, (v) a further increase in relative concentration of cinnamoylcocaine occurs in the woody material, (vi) berries contain the highest cinnamoylcocaine concentrations (up to 68% of the total of the two alkaloids), although there are significant variations within the berries themselves as there are in the leaves. A further conclusion, based on a comparison of the plants from the two geographical collections, is that the accessions available to us were readily distinguishable on the basis of the cocaine/cinnamoylcocaine ratios. It is likely that this is a valid method of subspecies distinction,[12] although the effects of other factors

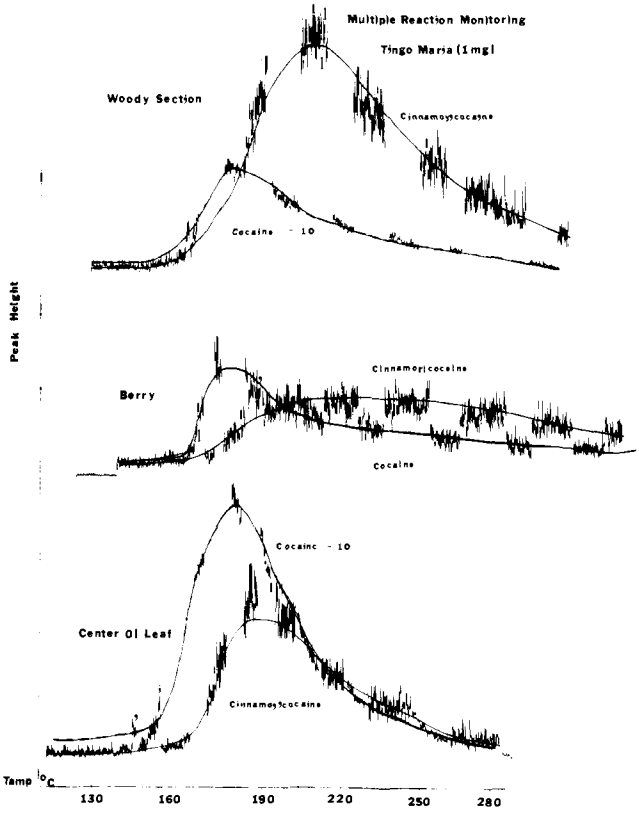

Figure 1. Vaporization profile of cocaine and cinnamoylcocaine in Tingo Maria by multiple reaction monitoring (cocaine, $304^+ \rightarrow 182^+$; cinnamoylcocaine, $330^+ \rightarrow 182^+$). The temperature was increased approximately linearly with time.

(genetic, maturation, temporal, etc.) were not followed. The accessions are also distinguished by the extent to which the relative concentrations varied with sampling site. Thus, Tingo Maria showed a cocaine/cinnamoylcocaine ratio which varied from 54 (leaf margin) to 0.5 (inside of berry), while the corresponding Trujillo ratios are 11 and 4.

Our observations seem to point to the accumulation of cinnamoylcocaine in older tissue such as stem vs. leaf. However, estimations of concentrations from total peak abundances using samples of known weight show that much of the observed variation in *relative* concentration is associated with changes in cocaine concentrations. This is some four times greater in the leaf than in the woody material and is smaller than either in the berry. The two accessions showed differences in both the alkaloid ratio (2% vs. 9% cinnamoylcocaine in powdered leaf) and in total alkaloid where the Tingo Maria had some five times the amount as did the Trujillo. These latter observations have obvious pharmacologic significance, considering that cinnamoylcocaine is much less active than cocaine.[13]

Applications of this methodology to animal tissues are being initiated. It should be noted that 100% recoveries from the tissue are not needed so long as the compounds of interest are not subject to selective losses. The difficulties of absolute analyses from a tissue matrix are circumvented here by employing a second and related molecule as internal standard.

It is emphasized that even the most sensitive of alternative methodologies of trace organic analysis assume a large initial sample. It is simply not possible to extract, derivatize, and chromatograph small plant tissue samples of the order of 1 mg as employed here. A clear advantage also exists in total analysis times.

Acknowledgment. This work was supported by the National Science Foundation (CHE 77-01295).

References and Notes

(1) T. L. Kruger, J. F. Litton, and R. G. Cooks, *Anal. Chem.*, **48**, 2113 (1976).
(2) R. W. Kondrat and R. G. Cooks, *Anal. Chem.*, **50**, 81A (1978).
(3) J. H. McReynolds and M. Anbar, *Anal. Chem.*, **49**, 1832 (1977).
(4) F. W. McLafferty and F. M. Bockhoff, *Anal. Chem.*, **50**, 69 (1978).
(5) These reactions have previously been shown to characterize specifically these alkaloids in coca leaf: R. W. Kondrat, G. A. McClusky, and R. G. Cooks, *Anal. Chem.*, **50**, 2017 (1978).
(6) D. Zakett, R. G. A. Flynn, and R. G. Cooks, *J. Phys. Chem.*, **82**, 2359 (1978).
(7) T. L. Kruger, R. G. Cooks, and J. L. McLaughlin, *J. Org. Chem.*, **42**, 4161 (1977).
(8) R. W. Kondrat, R. G. Cooks, and J. L. McLaughlin, *Science*, **199**, 978 (1978).
(9) Samples (1-mg total) were introduced in glass capillaries, and the probe temperature was raised from 70 to 280 °C at a rate of 20–40 °C/min. Isobutane was employed as chemical ionization reagent gas (0.25–0.35 Torr) and N_2 as collision gas (indicated pressure 4–5 × 10^{-5} Torr, estimated 1–2 × 10^{-3} Torr). The probe was cleaned with methanol between each run, and blank runs were occasionally made but showed no sample carry-over.
(10) Plant materials were obtained and identified by Dr. Carlton E. Turner, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Miss. 38677, under the auspices of the National Institute of Drug Abuse and through Gen. Alejandro Costa Spirgatis, Empressa Nacional de la Coca, Lima, Peru.
(11) The chemical ionization mass spectra show abundant ions corresponding to the protonated molecules at optimum probe temperatures. However, other abundant ions are present and we do not consider the mass spectral peak alone to sufficiently characterize the alkaloids.
(12) Similar taxonomical assignments are made by B. Holmstedt, E. Jaatmaa, K. Leader, and T. Plowman, *Phytochemistry*, **16**, 1753 (1977), using more classical analytical techniques.
(13) R. H. F. Manske, "The Alkaloids Chemistry and Physiology", Vol. 1, H. L. Holmes, Ed., Academic Press, New York, 1950.
(14) (a) Department of Chemistry; (b) Department of Medicinal Chemistry and Pharmacognosy.

M. Youssefi,[14a] R. G. Cooks,*[14a] J. L. McLaughlin[14b]
Department of Chemistry and Department of Medicinal Chemistry and Pharmacognosy
Purdue University, West Lafayette, Indiana 47907
Received March 26, 1979

The Structure of Asukamycin, a Possible Shunt Metabolite from 3-Dehydroquinic Acid in the Shikimate Pathway

Sir:

Asukamycin (**1**) is an antibiotic, produced by *Streptomyces nodosus* subsp. *asukaensis*, showing antimicrobial activity against Gram-positive bacteria as well as anticoccidial activity in chikens.[1] We here assign the structure of **1**, which is proved to have a naturally unique monosubstituted cyclohexane structure. Furthermore, the stereochemical features of **1** are also described since the stereochemistry of a related antibiotic manumycin (**2**),[2] elaborated by *Streptomyces parvus*, has not been reported.

Microanalysis and field desorption mass spectrometry established the molecular formula of **1** as $C_{31}H_{34}N_2O_7$ ($M^{+\cdot}$: *m/e* 546). The ^{13}C NMR signals at 52.4 ppm ($^1J_{C\text{-}H}$ = 190 Hz) and 56.4 ppm ($^1J_{C\text{-}H}$ = 187 Hz) were attributable to an epoxide, whose protons were observed in the 100-MHz spectrum at 3.64 ppm (d, J = 4.0 Hz, H-6) and 3.72 ppm (dd, J = 4.0 and 2.5 Hz, H-5), the latter being coupled to an olefinic proton at 7.40 ppm (d, J = 2.5 Hz, H-3). In addition, the ^{13}C NMR spectrum showed the signals of a ketone carbonyl at 189.2 ppm (s), two amide carbonyls at 165.7 ppm (s) and 164.7 ppm (s), and a tertiary carbinol carbon at 70.5 ppm (s).[3] In the ^1H NMR spectrum, two exchangeable protons and a highly deshielded exchangeable proton were observed at 8.02 and 13.6 ppm, respectively, and a broad singlet (H-4″ and H-5″) was displayed at 2.58 ppm. These diagnostic NMR data suggested the similarity of **1** to **2**.[2]

The partial structure A was implied by acetolysis (Ac_2O, 155 °C, 5 h) of **1** giving rise to **3**, mp 164–165 °, which was identified by the spectral data.[2,4]

Chromic acid oxidation (CrO_3 in 80% AcOH, room temp, 3 h) of **1** afforded **4**, $C_{19}H_{21}NO_4$ ($M^{+\cdot}$: *m/e* 327.1440 (found), 327.1471 (calcd), mp 176–180 °C, $[\alpha]_D^{22}$ +33.6° (*c* 0.96, $CHCl_3$), λ_{max}^{MeOH} 300 nm (log ε 4.10) and 347 nm (log ε 4.16). The IR bands (ν_{max}^{KBr} 3300, 1660, 1600, and 1490 cm^{-1}) and the ^{13}C NMR signal at 166.7 ppm (s) suggested a conjugated amide structure in **4**, and the prominent mass-spectral fragment ion at *m/e* 189 indicated the acid portion as $C_{13}H_{17}O$ (*m/e* 189.1242 (found), 189.1280 (calcd), and the amine portion as $C_6H_4NO_3$.[5] The structure of the latter was established by comparison of the NMR data of **4** with those of a manumycin derivative.[2] In the acid portion consisting of a carbonyl group, six -CH= groups and C_6H_{11}, the carbon signals of the C_6H_{11} residue observed at 26.3 ppm (t, 2 C), 26.5 (t), 33.0 (t, 2 C), and 41.6 (d) were consistent to those of a monosubstituted cyclohexane.[6] The remaining six -CH= groups must then be a triene, which was clarified by the 270-MHz ^1H NMR signals at 5.94 ppm (d, J = 14.8 Hz, H-2′), 7.38 (dd, J = 14.8 and 11.2 Hz, H-3′), 6.21 (dd, J = 11.2 and 14.8 Hz, H-4′), 6.62 (dd, J = 14.8 and 10.5 Hz, H-5′), 6.13 (dd, J = 10.5 and 15.4 Hz, H-6′), 5.95 (dd, J = 15.4 and 6.6 Hz, H-7′), and 2.02 (m, H-8′). These double bonds were accordingly determined to be all trans as shown in Figure 1. The cyclohexylmethylidene structure was further verified by nitric acid oxidation (70% HNO_3, 95 °C, 1 h) of **1** yielding cyclohexanecarboxylic acid **5**, the methyl ester of which was identified with an authentic specimen by the GC–MS analysis.

The $^{13}C\text{-}\{^1H\}$ NOE experiments established another partial structure of **1**.[7] Thus, irradiation of the ^1H NMR signal at 7.40 (H-3) or 3.70 ppm (H-5 and H-6) gave an intensity enhancement (90 and 92%, respectively) for the ^{13}C NMR signal at 70.5 ppm (C-4);[8] the partial structure B of **1** was proved.

The last six -CH= groups of **1** were consequently assigned to a conjugated triene connecting to the partial structures A and B. Since these olefinic proton signals in the 270-MHz ^1H

0002-7863/79/1501-3402$01.00/0 © 1979 American Chemical Society