(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).

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(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.
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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-{^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

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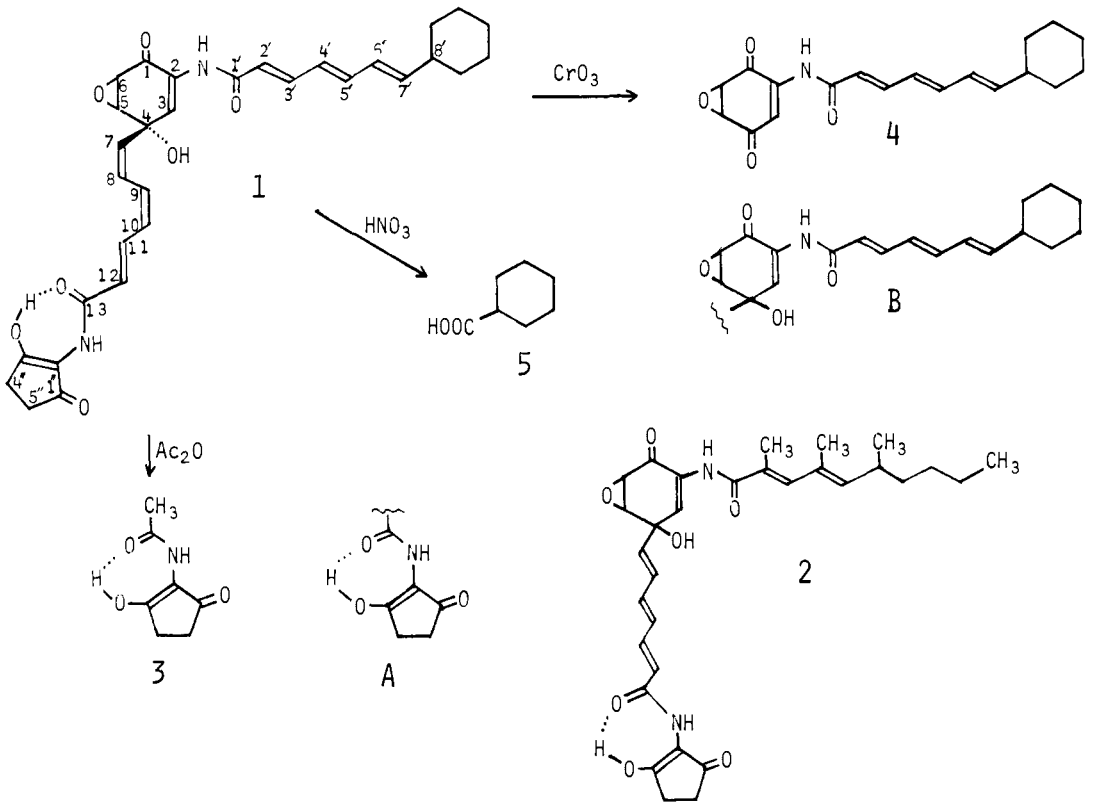

Figure 1. The structures of asukamycin (**1**), its degradation products, and manumycin (**2**).

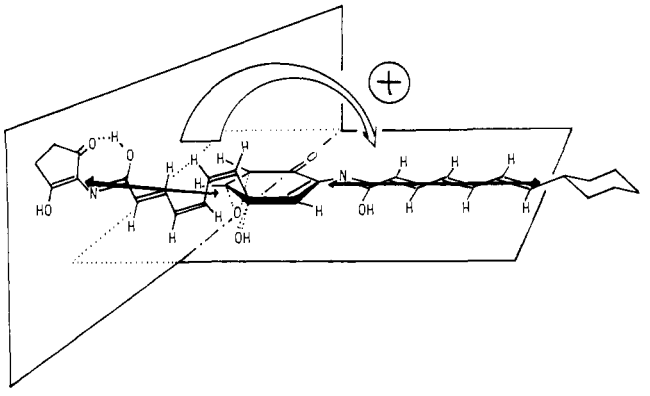

Figure 2. A stereochemical view of **1**. The amide bonds are delineated in tautomeric enol forms.

NMR spectrum were observed at 5.87 ppm (dd, J = 3.3 and 10.9 Hz, H-7), 6.60 (overlapped, H-8 and H-9), 6.42 (dt, J = 11.0 and 3.5 Hz, H-10), 7.33 (dd, J = 11.0 and 14.7 Hz, H-11), and 6.05 ppm (d, J = 14.7 Hz, H-12), the stereochemistry of the double bonds and the structure of **1** were as shown in Figure 1.

Among three asymmetric centers, the configuration of C-4 was deduced from the exciton chirality method.[9] The conformation of the central epoxycyclohexenone moiety was supposed to be almost planar since the W-type coupling was observed between H-3 and H-5 in **1** (^4J = 2.5 Hz) and in **4** (^4J = 2.4 Hz), and the chromophore in the partial structure B would be coplanar to the cyclohexenone plane. Therefore, the substituents on C-4 would be located above (β) or below (α) the said plane. If the other chromophore (C-7 through the partial structure A) is located above (β) the plane and the directions of the exciton dipoles are as shown in Figure 2, positive induced Cotton effects would be expected by the extended exciton chirality rule.[10] Since the CD spectrum (MeOH) of **1** showed the well-split intense Cotton effects, $\Delta\epsilon_{339}$ +39.0 and $\Delta\epsilon_{301}$ −49.2, the projection of the two chromophores should be clockwise and the *S* configuration was assigned for C-4. The epoxide stereochemistry is still under study.

Though the biosynthesis of **1** is not yet known, the origin of the central C_7N unit substituted by the probable polyketide chains can be analogous to that of ansamycins, which is reportedly derived from an intermediate in the shikimic acid pathway.[11] Significance of **1** is that the stereochemistry of C-4 is identical with that of 3-dehydroquinic acid (DHQ), and this may suggest that transamination to the C-3 carbonyl group of DHQ gives these C_7N units. Furthermore, it is worth noting that **1** (and presumably **2** as well) may be the first shunt metabolite derived from DHQ with retention of the tertiary carbinol moiety. Further investigations on the biosynthesis are in progress.

Acknowledgments. The authors are indebted to Professors J. A. McClosky, University of Utah, and C. C. Sweeley, Michigan State University, for field desorption mass spectrometry. Gratitude is also due to Professor T. Miyazawa, University of Tokyo, for the 270-MHz ^{1}H NMR spectrum and to Dr. J. Uzawa, The Institute of Physical and Chemical Research, for the ^{13}C-{^{1}H} NOE experiments.

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(4) Additional data of **3**; MS: *m/e* 155 ($M^{+\cdot}$) and 112; ν_{max}^{Nujol} 3250, 1600, and 1540 cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.20 (3 H, s), 2.56 (4 H, br s), 8.12 (1 H, br s), and 13.4 ppm (1 H, br s).
(5) NMR data for this portion; ^{13}C NMR ($CDCl_3$): 53.1 (d), 54.1 (d), 115.2 (d), 140.5 (s), 188.1 (s) and 192.4 ppm (s); ^{1}H NMR ($CDCl_3$): 3.92 (d, J = 3.6 Hz), 3.84 (dd, J = 3.6 and 2.4 Hz), 7.58 (d, J = 2.4 Hz) and 7.86 ppm (br s, exchangeable).
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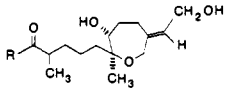
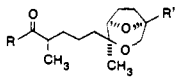
Zoapatanol and Montanol, Novel Oxepane Diterpenoids, from the Mexican Plant Zoapatle (*Montanoa tomentosa*)

Sir:

A tea prepared from the leaves of zoapatle (*Montanoa tomentosa*) has been used in Mexico for the past four centuries to induce menses and labor and terminate early pregnancy. Although there are many conflicting references[1] to the biological activity of zoapatle extracts, definitive studies leading to the isolation and identification of the biologically active component(s) of this plant have not yet been reported.[2,3] We describe herein our efforts that have culminated in the structural elucidation of two novel and biologically active oxepane diterpenoids, zoapatanol (**1**) and montanol (**2**).





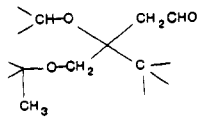
1 R = $(CH_3)_2C{=}CH{-}CH_2{-}$

2 R = $(CH_3)_2CH{-}C(CH_3){=}C(H){-}$

3 R = $(CH_3)_2C{=}CH{-}CH_2{-}$ R' = CH_2CHO

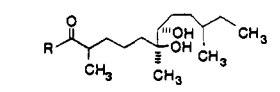
4 R = $(CH_3)_2C{=}CH{-}CH_2{-}$ R' = $CH{=}CH_2$

Aqueous extraction of the leaves, followed by treatment with organic solvents, afforded a crude extract possessing contragestational activity.[4] Chromatography of this extract first on a silica gel column and then on a vinyl acetate copolymer column afforded **1** and **2** as oils.[5] Although **1** had an apparent M^+ of 320 by electron ionization–mass spectroscopy (EI-MS), it became clear from the preparation of a variety of derivatives that the true M^+ was 338 ($C_{20}H_{34}O_4$), which was confirmed by chemical ionization–mass spectroscopy (CI-MS). Twenty carbon atoms were observed by ^{13}C NMR and from examination of the chemical shifts and multiplicities in the ^{13}C NMR and by decoupling experiments in the ^1H NMR, the following were identified: $\delta_{Me_4Si}^{CDCl_3}$ 5.47 (t, J = 7 Hz, 1 H, $>C{=}CHCH_2C(=O)-$), 5.29 (t, J = 7 Hz, 1 H, $>C{=}CHCH_2OH$), 4.14 (d, J = 7 Hz, 2 H, $>C{=}CHCH_2OH$), 4.08 (s, 2 H, $-COCH_2C{=}$), 3.53 (d, d, J = 4, 8 Hz, 1 H, $>CHOH$), 3.12 (d, J = 7 Hz, 2 H, $>C{=}CHCH_2C(=O)-$), 1.75 and 1.62 (s, 3 H each, $(CH_3)_2C{=}CH$), 1.14 (s, 3 H, $>C(CH_3)OCH_2-$), and 1.08 (d, J = 7 Hz, 3 H, CH_3CH-). Treatment of **1** with MnO_2 in CH_2Cl_2 gave the saturated aldehyde **3**, presumably arising by intramolecular addition of a secondary alcohol to the initially formed product.[6] The assignment was supported by: M^+ 336, the absence of an OH in the IR, and the appearance of a triplet at δ 9.73 coupled to a doublet at δ 2.60 (J = 2 Hz) for a CH_2CHO group. In addition, the methylene at δ 4.08 was absent and replaced by an AB q at δ 3.29 and 3.76 (J = 11 Hz). These data were consistent with the following partial formula:

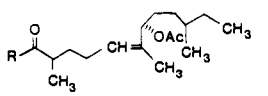


Analysis of the spectral data for the dehydrated product **4** obtained when **1** was treated with *p*-TsOH in benzene indicated that a similar type of intramolecular reaction had occurred: M^+ 320;[7] δ 3.29 and 3.75 (AB q, 2 H, J = 11 Hz, $-OCH_2C-$) and 5.0–6.2 (3 H, $-OCCH{=}CH_2$).

Hydrogenation (PtO_2, $NaNO_2$)[8] of **1** saturated both double bonds, whereas hydrogenation over Pd/C led to the uptake of 4 mol of H_2 and the isolation of a major product ($C_{20}H_{40}O_3$) in which both double bonds were saturated, hydrogenolysis of the two allylic oxygen bonds had occurred, and the secondary alcohol was still intact [δ 3.26 (m, 1 *H*, $>CHOH$)]. The vicinal nature of the diol in the hydrogenation product was substantiated by monoacetylation (Ac_2O, pyr, room temperature) followed by dehydration ($POCl_3$, pyr, room temperature) to an allylic acetate **6** [δ 1.59 (s, 3 H, $CH_3C{=}$), 5.1 (m, 1 H, $=CHOAc$)]. An unequivocal structure proof for **5** was obtained when MnO_2 cleavage[9] of **5** afforded the diketone **7** and aldehyde **8** (Scheme I). In addition to spectral data supporting their structures, both compounds were synthesized and their identities confirmed. The synthesis of **7** is outlined in Scheme II, while the known aldehyde **8**[10] was prepared by treatment of 4-methyl-1-hexene with 9-BBN followed by oxidation (CrO_3, pyr, CH_2Cl_2). These results, along with chemical, spectroscopic, and biogenetic considerations on a variety of derivatives,[11] led us to propose an oxepane derived structure **5** for the hydrogenation product.



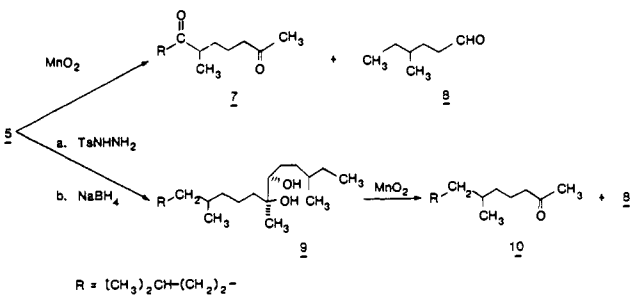
5 R = $(CH_3)_2CH{-}(CH_2)_2-$



6 R = $(CH_3)_2CH{-}(CH_2)_2-$

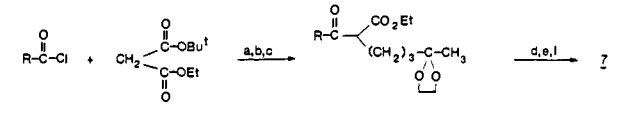
The same aldehyde **8** was obtained along with hexahydropseudoionone (**10**)[12] when **5** was converted to a tosylhydrazone and reduced with $NaBH_4$ to the deoxo-*vic*-glycol **9**

Scheme I



R = $(CH_3)_2CH{-}(CH_2)_2-$

Scheme II



R = $(CH_3)_2CH{-}(CH_2)_2-$

a. Mg, EtOH, ether; b. TsOH, Δ, PhH; c. NaH, DMF, $CH_3{-}C(O_2)(CH_2)_3{-}Cl$; d. MeI, DMF, NaH; e. NaOH, EtOH; I. TsOH, Δ, acetone

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