tricyclic hydrophobic moiety as compared with the tetracyclic hydrophobic moiety of I that, in turn, may account for the higher energies of interaction found for I.

Based on the intercalation model to describe the binding of acridines to DNA by insertion between adjacent base pairs (16–18), it recently was proposed (19) that, in the interaction of I with nucleic acids, the positive charge of the amino sugar associates through electrostatic forces with negatively charged phosphate groups of the DNA chain while the tetracyclic hydrophobic moiety, through van der Waals interactions, inserts itself between adjacent base pairs. The binding energy of the acridines to double-stranded DNA is about 6–10 kcal/mole (20). The binding energy of I to double-stranded DNA, recently estimated by using the values of the association constants measured with spectroscopic methods (19), is about 7–12 kcal/mole.

The results presented here seem to indicate that the same type and order of magnitude of van der Waals forces are in effect in the interaction of I and II with phospholipid monolayers.

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# $^{13}$C-NMR Spectroscopy of Tropane Alkaloids


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Received June 28, 1977, from the *Institut für Pharmazeutische Chemie der Westfälischen Wilhelms-Universität, 58-62 Hittorfstr., D-4400 Münster (Westf), West Germany.* Accepted for publication September 14, 1977. *Present address: Faculty of Pharmacy, University of Assiut, Assiut, Egypt.



**Abstract** □ The natural abundance $^{13}$C-NMR spectra of tropine, atropine, scopolamine, cocaine, atropine methonitrate, and *dl*-tropic acid were determined at 22.63 MHz. With the aid of proton decoupling techniques and by comparison with analogous simpler compounds, it was possible to make self-consistent and unambiguous assignments of all carbon resonances for these alkaloids. Some important chemical shift trends were observed and should be useful in the identification of similar systems.



**Keyphrases** □ Tropane alkaloids, various—$^{13}$C-NMR spectra determined, carbon resonances assigned □ Alkaloids, various tropane—$^{13}$C-NMR spectra determined, carbon resonances assigned □ $^{13}$C-NMR spectroscopy—various tropane alkaloids, spectra determined, carbon resonances assigned


The proton magnetic resonance (PMR) spectra of alkaloids often are too complex to be useful in the structure elucidation of this large class of naturally occurring compounds. The complexity results from extensive spin–spin coupling among protons, overlap of numerous resonance patterns, line broadening arising from intermolecular association and/or $^{14}$N-quadrupolar relaxation effects. Natural abundance $^{13}$C-NMR spectroscopy has been especially useful in such cases (1).

The tropane alkaloids have been well characterized by structural and stereochemical investigations (2). However, only one limited $^{13}$C-NMR study, concentrating on the nonaromatic part of atropine, has been reported (3). The undertaking of a more detailed study was stimulated during the examination of the stability of tropane alkaloids in organic solvents when, consequently, need arose as to the structure elucidation of closely similar compounds. In addition, the broad acceptance of $^{13}$C-NMR spectroscopy as a new powerful tool of structural analysis awaits only the accumulation of chemical shift data on compounds representative of all types of natural products.

This report concerns the application of pulse and Fourier transform $^{13}$C-NMR techniques to structure assignments of tropane alkaloids.

## EXPERIMENTAL

**$^{13}$C-NMR Spectra**—$^{13}$C-NMR spectra were determined at 22.63 MHz in the Fourier transform mode using a spectrometer[1] interfaced with a computer system[2]. The spectrometer features field stabilization *via* internal deuterium lock. Alkaloidal bases were dissolved in chloroform-*d*, and tropic acid was dissolved in methanol-$d_4$; either a dimethyl sulfoxide-$d_6$ or deuterated methanol–deuterium oxide mixture (6:4) was used

[1] Brucker W-90 pulse.
[2] Brucker-Nicolet B-NC-12.

Table I—$^{13}$C-Chemical Shifts for Tropane Alkaloids in Deuterochloroform [a] (Nonaromatic Moiety)

| Carbon Identification | Multiplicity [b] | Tropine | Atropine | Scopolamine | Atropine Methonitrate [b] | Cocaine |
|---|---|---|---|---|---|---|
| 1 | d | 59.7 | 59.2 | 57.3 | 64.5 | 64.5 |
| 2 | t | 38.8 | 35.8* | 30.2 | 32.1 | 49.9 (d) |
| 3 | d | 63.3 | 67.4 | 66.3 | 67.9 | 66.6 |
| 4 | t | 38.8 | 35.6* | 30.4 | 32.1 | 35.2 |
| 5 | d | 59.7 | 59.2 | 57.3 | 64.5 | 61.3 |
| 6 | t | 25.5 | 24.7* | 55.4* (d) | 24.6* | 25.0 |
| 7 | t | 25.5 | 25.05* | 55.8* (d) | 24.7* | 25.0 |
| $NCH_3$ (9) | q | 39.8 | 39.7 | 41.6 | 44.4 (a) | 40.7 |
| $NCH_3$ (10) | q | — | — | — | 51.2 (e) | — |
| $OCH_3$ | q | — | — | — | — | 50.8 |
| C=O | s | — | — | — | — | 170.2 |

[a] Chemical shifts are in parts per million downfield from tetramethylsilane. Starred (*) values may be interchanged for the same compound. [b] Signal multiplicity obtained from SFORD spectra: s = singlet, d = doublet, t = triplet, and q = quartet; (a) = axial orientation, (e) = equatorial orientation.

as the solvent for atropine methonitrate. All samples were contained in precision-ground 10-mm o.d. tubes.

The spectra were obtained at ambient temperature by using the deuterium resonance of the solvent as the lock signal. The carbon signal of solvents provided the internal reference for chemical shift measurements, taking the frequency of the central peaks of the deuterated chloroform triplet as 1742.51 Hz (*i.e.*, 77.00 ppm downfield relative to tetramethylsilane), of the deuterated methanol septet as 1065.873 Hz (47.10 ppm), and of the dimethyl sulfoxide-$d_6$ septet as 895.672 Hz (39.58 ppm). In some runs, 5% tetramethylsilane was also added as an additional reference for control of the solvent signal. The chemical shifts reported are accurate to within 0.03 ppm.

For each compound, two spectra were determined. In one, all proton lines were decoupled by a broad band (~2.5 kHz) irradiation from an incoherent 90-MHz source (noise-modulated total proton decoupling). In the other run, a single-frequency off-resonance decoupling (SFORD) spectrum was made. Interferograms were stored in 8K (8000 channels) of computer memory with 8K output data points in the transformed-phase corrected real spectrum, and the chemical shifts were determined on 6000-Hz sweepwidth spectra. The typical pulse width was 2.5 μsec and the delay time between pulses was 1.2 sec, with a phase-alternating pulse sequence to eliminate residual noncoherent system noise echo effects.

Acquisition times averaged 1–2 over 8K data points for 0.5–0.8 *M* concentrations. Double this time was required for SFORD spectra.

**Chemicals**—Atropine and tropine bases, as well as *dl*-tropic acid and atropine methonitrate, were obtained commercially in analytical grade[3] and required no further purification. Cocaine and scopolamine bases were prepared from the salts obtained in pharmaceutical grade[3] by employing standard procedures (4). The melting points and $[\alpha]_D$ values of these prepared bases agreed with literature values.

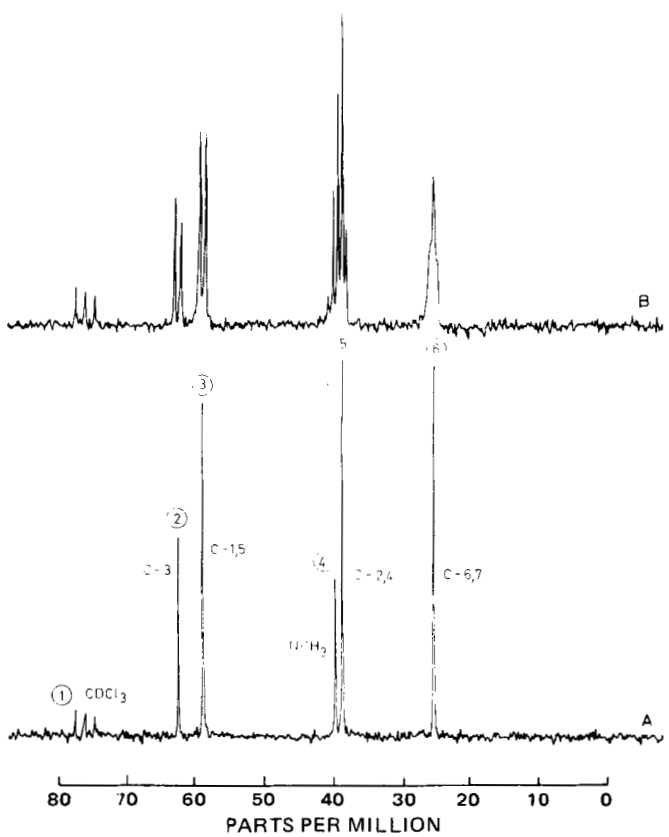
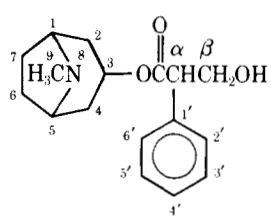



**Figure 1**—$^{13}$C-NMR spectra of tropine in deuterated chloroform. Key: A, broad band decoupled spectrum; and B, off-resonance decoupled spectrum.

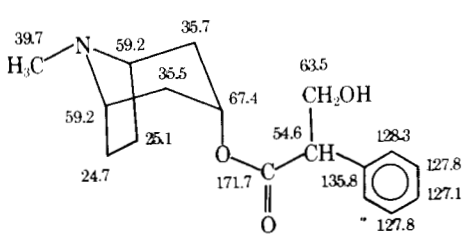
## RESULTS AND DISCUSSION

Most of the $^{13}$C-NMR chemical shift assignments for the tropane alkaloids (Tables I and II) were based on empirical correlations and the principle of additivity of substituent effects, which have been summarized in reported equations (5, 6). Although these equations were based on simple paraffins, the qualitative trends found for the $^{13}$C-shifts in paraffins have been extended to predict the relative order of $^{13}$C-chemical shifts in other compounds (7–9). The most important of these trends are: (*a*) that substitution of a carbon (or a more electronegative atom or group) for a directly attached hydrogen produces a downfield shift [*i.e.*, the more carbons alpha to the carbon in question, the more downfield the $^{13}$C-shift ($\alpha$-effect)]; (*b*) that substitution of a carbon or other atom for a hydrogen attached to a carbon alpha to the carbon in question also produces a downfield shift [*i.e.*, the more substitution at the carbon alpha to the carbon in question, the more downfield the $^{13}$C-shift ($\beta$-effect)]; and (*c*) that hydrogens or other groups attached to carbons gamma to the carbon in question produce an upfield shift ($\gamma$-effect). The $\alpha$- and $\beta$-effects are presumably through bond effects while the $\gamma$-effect is a steric compression effect (10).

The numbering system for tropane alkaloids is shown in Structure I.




I




II

[3] Merck, Darmstadt, German Federal Republic.

Table II—$^{13}$C-Chemical Shifts for Tropane Alkaloids [a] (Aromatic Moiety)

| Carbon Identification | Multiplicity | Atropine | Scopolamine | Atropine Methonitrate | Tropic Acid | Cocaine |
|---|---|---|---|---|---|---|
| 1′ | s | 135.9 | 135.4 | 136.0 | 136.7 | 130.2 |
| 2′ | d | 128.3 | 128.3 | 129.7 | 128.1 | 127.9 |
| 3′ | d | 127.8 | 127.6 | 128.9 | 128.6 | 129.3 |
| 4′ | d | 127.1 | 127.3 | 128.9 | 127.1 | 132.5 |
| 5′ | d | 127.8 | 127.6 | 128.9 | 128.6 | 129.3 |
| 6′ | d | 128.3 | 128.3 | 129.7 | 128.1 | 127.9 |
| C=O | s | 171.6 | 171.2 | 173.4 | 175.2 | 165.5 |
| α-CH | d | 54.6 | 54.0 | 54.3 | 54.7 | — |
| β-$CH_2OH$ | t | 63.4 | 63.4 | 64.5 | 64.0 | — |

[a] See footnote of Table I.

The $^{13}$C-chemical shifts for the alkaloids are shown in Tables I and II. Some spectra are illustrated in Figs. 1–5, while the chemical shifts for atropine are indicated on its formula in Structure II. In each case, noise-decoupled and SFORD spectra were obtained. The former (broad band decoupling) yielded a single line signal for each carbon atom type with peak intensity roughly proportional to the number of carbons; the multiplicities generated in the SFORD spectra enabled the distinction between methyl (quartet), methylene (triplet), methine (doublet), and quaternary (including carbonyl) (singlet) carbon resonances.

The $^{13}$C-resonances of the tropane alkaloids can be grouped into two main regions corresponding to the tropane ring and the aromatic acid moiety of the molecules (Tables I and II).

**Tropane Ring**—In tropine, there are five separate signals in the 20–65-ppm $^{13}$C-chemical shift region relative to tetramethylsilane (Table I and Fig. 1). The symmetry-induced structural simplicity of tropine rendered it an easy object for assignment and facilitated later assignments of more complex molecules. The farthest downfield signal corresponds to C-3 carrying the hydroxyl group, while the *N*-methyl carbon is easily assigned the 39.8-ppm peak on the basis of SFORD multiplicity as well as peak intensity. The overlapping triplet at 38.8 ppm is due to C-2 and C-4, while the poorly resolved triplet farthest upfield is assigned to C-6 and C-7. The remaining methine signals at 59.7 ppm obviously belong to the C-1 and C-5 identical carbons. Evaluation of these signals was based on the additivity of substituents previously mentioned as well as comparison with β-methylene shift values of piperidine, pyrrolidine, and their *N*- and *C*-methyl derivatives (11–13) and the expected α-alkyl substituent effects (7, 8, 14, 15).

The weak shielding of C-2 and C-4 and the strong upfield shift of C-6 and C-7 indicate that the *N*-methyl group in tropine is oriented equatorially relative to the piperidine ring. The spectrum of atropine methonitrate (Table I) shows two distinct *N*-methyl signals, the upfield one corresponding to axial orientation and the lower field signal corresponding to equatorial orientation. The spectrum of this quaternary salt also exhibits a general downfield shift of most signals, amounting to 5 ppm for the α-carbon and 10 ppm for the equatorial methylene signal.

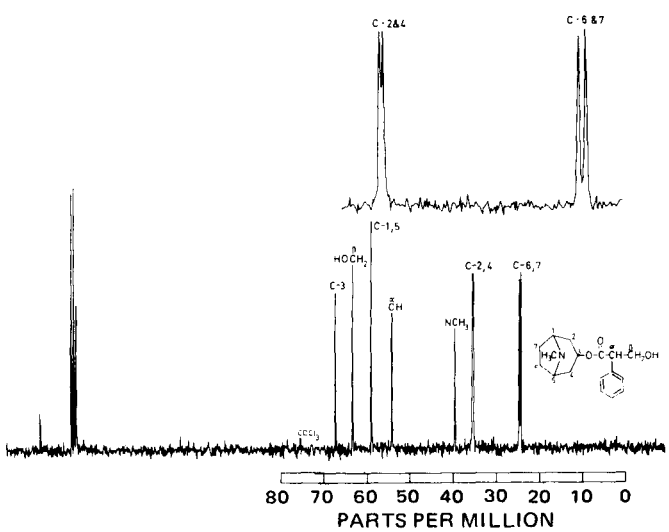



**Figure 2**—$^{13}$*C-NMR broad band decoupled spectrum of atropine in deuterated chloroform. The upper curve is a scale expansion of the signals of C-2, C-4 and C-6, C-7.*

Therefore, the quaternized salts should be useful as diagnostic derivatives for $^{13}$C-NMR analysis similar to the *N*-oxides (3) in view of the strong chemical shift changes. Unfortunately, however, different solvents are usually required for the free alkaloid and the salt so solvent effects are superimposed upon the derivative effects.

That $^{13}$C-NMR spectroscopy is a more powerful and useful tool than PMR spectroscopy, especially in revealing subtle structural details, is illustrated by the splitting of the signals for C-6 and C-7 as well as for C-2 and C-4 in atropine into fine doublets in comparison with the singlets observed in the unesterified tropine (Fig. 2). This observation suggests unsymmetrical orientation of the tropic acid moiety, probably due to hydrogen bond formation between the hydroxyl group of this acid and its carbonyl function and the general inclination of the ester group at C-3 to the lower plane of the azabicyclo ring. The same trend is also found in the spectrum of scopolamine (Fig. 3) but not in the signal of C-6 and C-7 in cocaine in which the aromatic moiety lacks the hydroxyl group. The obvious future application of this revealing of fine structural details would be in the possible greater understanding of the bioeffects of these

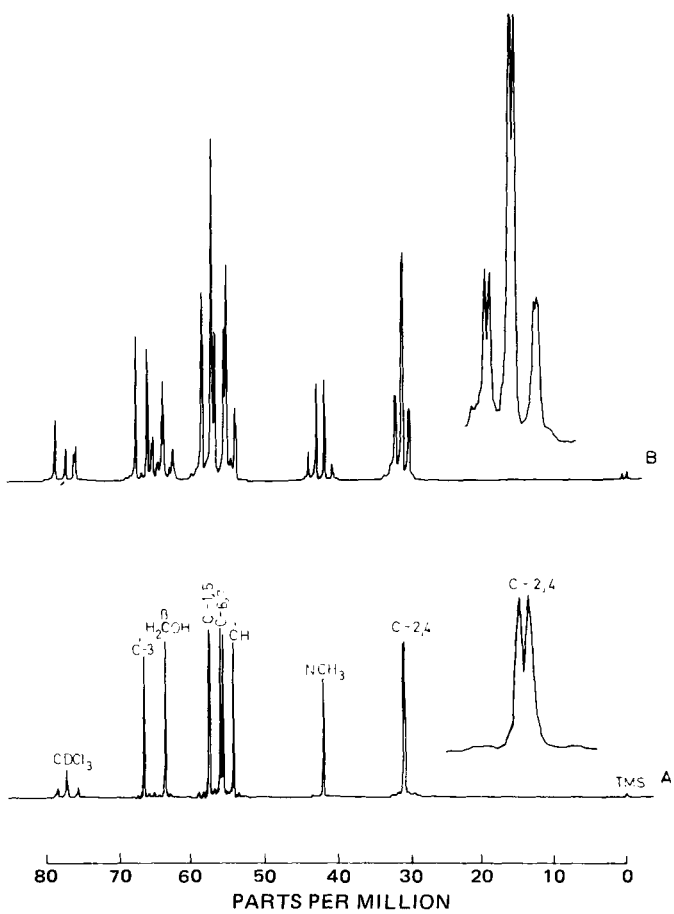



**Figure 3**—$^{13}$*C-NMR spectra of scopolamine (nonaromatic region) in deuterated chloroform. Key: A, broad band decoupled spectrum; and B, off-resonance decoupled spectrum. The signals for C-2 and C-4 are shown expanded on the right-hand side of both spectra.*

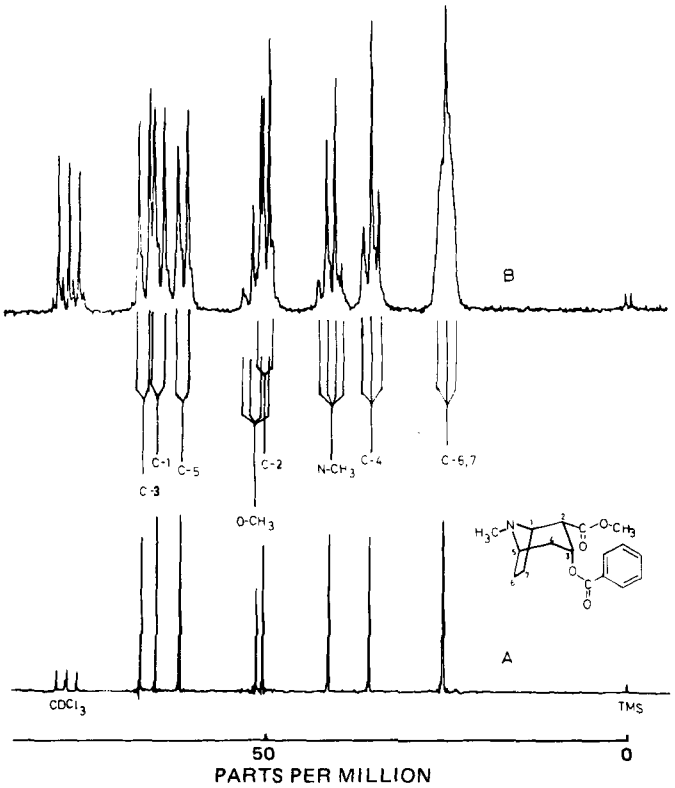



**Figure 4**—$^{13}C$-*NMR spectra of cocaine (nonaromatic region) in deuterated chloroform. Key: A, broad band decoupled spectrum; and B, off-resonance decoupled spectrum.*

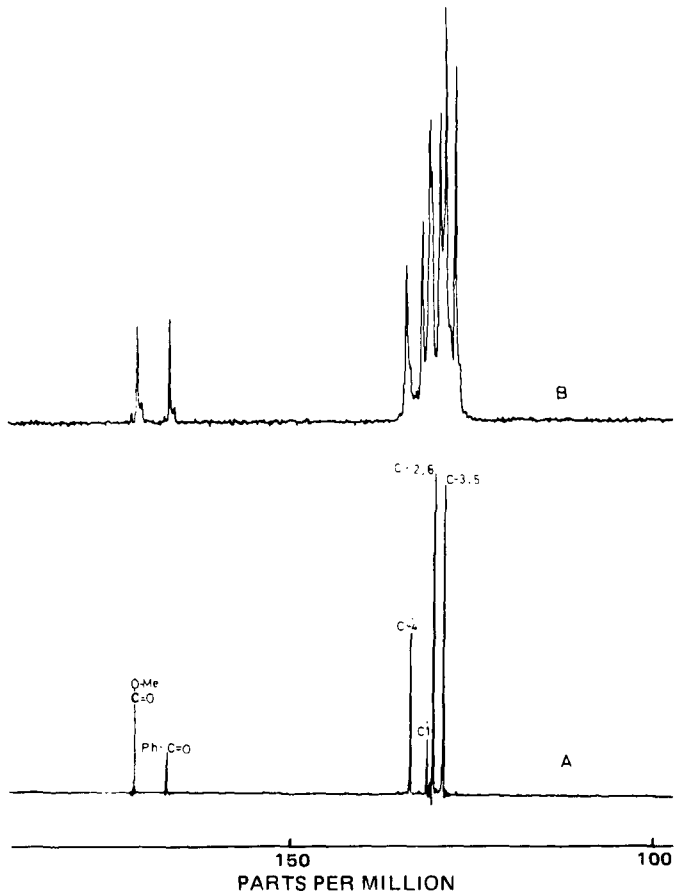



**Figure 5**—$^{13}C$-*NMR spectra of cocaine (aromatic region) in deuterated chloroform. Key: A, broad band decoupled spectrum; and B, off-resonance decoupled spectrum.*

compounds based on a closer knowledge of molecular orientation at receptors and in body fluids. The assignment of the $\alpha$-CH and $\beta$-$CH_2OH$ carbons of atropine, scopolamine, and atropine methonitrate was based on SFORD multiplicity as well as inspection of the spectra of the separate moieties: tropine and tropic acid (Tables I and II).

The lack of symmetry of the ecgonine moiety of cocaine makes its $^{13}C$-NMR analysis more difficult. However, the simpler aryl moiety, as well as the $^{13}C$-NMR effects observed among *N*- and *C*-methyl piperidines and piperidones (11–13), aided in the shift assignments. Substitution of the ester group at C-2 for hydrogen brings about a considerable downfield shift of the $^{13}C$-signal of this carbon (Table I and Fig. 4), amounting to a 14-ppm lower field than the corresponding carbon in atropine, and the SFORD multiplicity is now the expected doublet.

The assignment of signals for C-6, C-7, and C-4 was straightforward based on comparison with other alkaloids and SFORD spectra. The differentiation between the two quartets due to *O*- and *N*-methyl groups was based on reported observations on analogous systems (1–8), where the lower field peaks were assigned to carbons bonded to oxygen. Generally, a carbon bonded to nitrogen resonates about 10-ppm higher field than a similarly substituted carbon bonded to oxygen (1, 8, 14). Consideration of $\alpha$- and $\beta$-effects, as well as comparison with other tropanes, aided in the assignment of the most downfield signal of ecgonine to C-3 and the second and third signals higher field to C-1 and C-5, respectively (Fig. 4 and Table I). All three carbons, however, have the same SFORD multiplicity.

**Aromatic Moiety**—The aromatic region of the spectra of tropyl and benzoyl esters could be assigned without difficulty from the carbon-to-hydrogen coupling patterns (SFORD multiplicity) and intensities of peaks, as well as the alternating shielding and deshielding effect of the substituent at C-1′ (Table II). Thus, the carbon with the substituent (C-1′) did not show the 160-Hz directly bonded carbon–hydrogen coupling. The *para*-carbon peaks had only half the intensity of either *ortho*- or *meta*-carbons. The latter could be distinguished from each other by the difference in the spectral pattern arising from long range CH coupling (16). The electron-donating $CH_2OH$ substituent in tropic acid showed the expected alternating shielding and deshielding effect on the even- and odd-numbered carbons, respectively. This result aided in assigning the farthest upfield signal in the aromatic region of tropic acid (Table II) to C-4′, the next downfield peak to C-2′ and C-6′, and the remaining methine signals to C-3′ and C-5′. The single carbonyl carbon of tropic acid was easily assigned the farthest downfield signal. It is shifted some 4 ppm upfield in the esterified alkaloids relative to the unsubstituted acid, following the general trend found among carboxylic acid and esters (7–9, 16).

This order of aromatic carbon signals is reversed in the benzoyl moiety of cocaine (Fig. 5) because of the reverse in the shielding parameters of aromatic carbons induced by the directly attached electron-abstracting carbonyl group. Thus, the even-numbered carbons are deshielded more than the odd-numbered ones. This result aided in assignment of the half-intense methine signal at 132.5 in the cocaine spectrum to C-4′ and the singlet at 130.2 to C-1′. The next upfield twice-intense signals were assigned to C-2′, C-6′, and C-3′, C-5′, respectively. The differentiation of the two carbonyl carbons of cocaine (Tables I and II and Fig. 5) was based on substitution parameters (8) and comparison with similar systems (17).

## CONCLUSION

Because of the recent advances in instrumentation, $^{13}C$-NMR spectroscopy is becoming an increasingly useful tool in structural investigation and compound identification. The substituent additivity parameters utilized in this work proved again that empirical schemes are most important in the prediction of structure from $^{13}C$-NMR spectra and encourage the expansion of the data bank to include all possible structural variations. Many subtle and important structural features could be revealed by simple inspection of spectra. In comparison, the PMR spectrum of simple methyl benzoate required complete computer simulation (18). This evaluation of $^{13}C$-NMR shifts of a limited group of alkaloids should be a useful contribution to this expanding field of structural elucidation.

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# Colorimetric Determination of Catecholamines by 2,3,5-Triphenyltetrazolium Chloride

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Received May 16, 1977, from the *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Assiut, Assiut, Egypt.* Accepted for publication September 22, 1977.

**Abstract** □ A convenient spectrophotometric method was developed for the determination of epinephrine, levarterenol, isoproterenol, and methyldopa by reduction of 2,3,5-triphenyltetrazolium chloride and subsequent measurement of the formazan at 485 nm. With absolute alcohol as the solvent, maximum color absorption was attained in 30 min at 25° in the presence of 0.1 *N* KOH. Evidence is provided to account for the reduction of the tetrazolium salt at the expense of the epinephrine catechol moiety. In addition to the considerably high values of the molar absorptivities of the chromogen formed, ideal adherence of the color absorption to the Beer–Lambert law permitted a sensitive microdetermination of these catecholamines in both pure forms and pharmaceutical formulations. The tetrazolium interaction was selective. No interference was encountered from common catecholamine antioxidants, adjuvants, or noncatechol degradation products.

**Keyphrases** □ Epinephrine—colorimetric analysis in bulk drug and dosage forms □ Levarterenol—colorimetric analysis in bulk drug and dosage forms □ Isoproterenol—colorimetric analysis in bulk drug and dosage forms □ Methyldopa—colorimetric analysis in bulk drug and dosage forms □ Colorimetry—analyses, epinephrine, levarterenol, isoproterenol, and methyldopa in bulk drug and dosage forms □ Catecholamines, various—colorimetric analyses in bulk drug and dosage forms □ Adrenergic agents—epinephrine, levarterenol, and isoproterenol, colorimetric analyses in bulk drug and dosage forms □ Antihypertensives—methyldopa, colorimetric analysis in bulk drug and dosage forms

The presence of various catecholamine congeners in many pharmaceutical formulations necessitates a rapid, economical, and sensitive analytical method. Most of these derivatives are officially assayed by chemical, titrimetric, and spectrophotometric procedures (1, 2). Because of their phenolic and basic functions, catecholamines interact with many chromogenic reagents such as potassium ferricyanide–ferric chloride (3), alkali ferrocyanates (4), *p*-nitrophenyldiazonium chloride (5), nitrosomercurials (6), and alkali molybdates (7). Other earlier reported chromogens include iodine, ferric ion, nitrous acid, and 1,2-naphthoquinone 4-sodium sulfonate (8). However, accurate colorimetric determinations of pharmaceutical catecholamines using their reducing potential have not been developed.

The present study investigated 2,3,5-triphenyltetrazolium chloride as a convenient reagent for the colorimetric determination of epinephrine, levarterenol, isoproterenol, and methyldopa, both in pure forms and dosage formulations. The fact that tetrazolium salts can be reduced selectively into highly colored formazan dyes constituted the basis of the current work (9).

## EXPERIMENTAL

**Instrumentation**—A double-beam spectrophotometer[1], a pH meter[2] fitted with a sealed calomel electrode, a shielded glass electrode, and a suitable thermostated[3] water bath were used.

**Catecholamines**—Pharmaceutical grade epinephrine hydrochloride, levarterenol bitartrate, isoproterenol hydrochloride, and methyldopa were utilized as the working standards.

**Catecholamine Dosage Forms**—The following commercial formulations were analyzed: methyldopa tablets[4], epinephrine injection[5], procaine–epinephrine injection[6], levarterenol injection[7], and isoproterenol solution[8].

**Reagents**—*Tetrazolium Solution*—Dissolve 0.5 g of pure 2,3,5-tri-

[1] Spektromom-203, MOM, Budapest, Hungary.
[2] Radelkis OP-401/2, Budapest, Hungary.
[3] T-606 MTA, Budapest, Hungary.
[4] Contains 250 mg of methyldopa/tablet; El-Kahira-MSD, Cairo, Egypt.
[5] Contains 1.0 mg of epinephrine/1-ml ampul; Misr Co. for Pharmaceuticals, Cairo, Egypt.
[6] Contains 10.0 μg of epinephrine and 10.0 mg of procaine hydrochloride/1-ml ampul; El-Nile Co. for Pharmaceuticals, Cairo, Egypt.
[7] Contains 1.0 mg of levarterenol/1-ml ampul; El-Nile Co. for Pharmaceuticals, Cairo, Egypt.
[8] Isoprenaline, contains 10.0 mg of isoproterenol hydrochloride/1 ml of solution; El-Nile Co. for Pharmaceuticals, Cairo, Egypt.