Configuration of Dienestrol

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Abstract □ Uncertainty concerning the configuration of dienestrol was resolved by a detailed spectrochemical investigation, including single-crystal X-ray diffraction, of the active drug and its stereoisomers. A symmetric structure in which the phenyl and methyl groups are *cis* about each double bond is unambiguously assigned.

Keyphrases □ Dienestrol—determination of configuration, single-crystal X-ray diffraction □ Estrogens—determination of configuration of dienestrol, X-ray diffraction □ X-ray diffraction—determination of dienestrol configuration

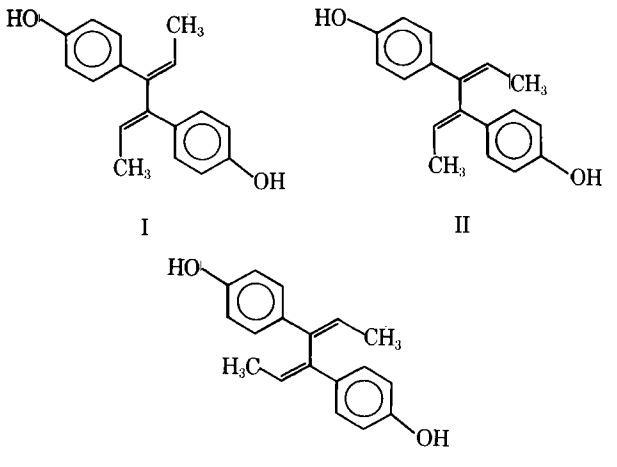
The synthetic estrogen dienestrol [3,4-bis(*p*-hydroxyphenyl)-2,4-hexadiene] was originally prepared in 1939 (1). Its exact structure, however, has remained a controversial subject (2–5); the configuration remains unspecified in NF XIV (6).

Theoretically, there are three possible stereoisomers, I–III. Koch and coworkers (2, 3) suggested configuration I for the isomer available to them. Later, presumed authentic samples of each stereoisomer were synthesized (4, 5); their corresponding diacetates were prepared by dehydration of mixtures of *meso*- and racemic 3,4-bis(*p*-acetoxyphenyl)-3,4-hexanediols. On the basis of the IR absorption of the diacetates, Lane and Spialter (5) concluded that the drug dienestrol has steric configuration III.

In view of the authors' interest in the stereochemistry and photoreactivity of synthetic estrogens (7), the question of the structure of dienestrol was reexamined from five distinct points of view: NMR spectroscopy, UV spectrophotometry, molecular models, chemical reactivity, and X-ray diffraction. Results prove that dienestrol has Structure I.

EXPERIMENTAL

Dienestrol was used as received from the supplier[1] without further purification, except that the specimen for X-ray crystallography was recrystallized from absolute alcohol, mp 227–228°.



[1] K&K Laboratories, Plainview, N.Y.

NMR spectra[2] were obtained in acetone-d_6 with tetramethylsilane internal standard.

Electronic absorption spectra were obtained in 95% ethyl alcohol on a recording spectrophotometer[3]; 1-cm silica cells were used.

X-ray crystallography was performed on an automated single-crystal X-ray diffractometer[4]. Preliminary investigation of the space group and cell dimensions were made by means of oscillation and Weissenberg photographic techniques. The accurate cell dimensions and intensities were collected on the X-ray diffractometer by the θ–2θ scan technique. All calculations for the preliminary data treatment, solution by direct methods, and refinement by full matrix least squares of the structure and the calculations of bond lengths and angles were carried out using XRAY72 (8) on a computer[5]. Figure 1 was produced using the ORTEP (9) crystallographic plotting program.

The structure determination was carried out using the NORMSF, SINGEN, PHASE, and FOURR links of the XRAY72 system of crystallographic programs. The E map produced revealed the molecule, including hydrogen atoms lying on a center of symmetry at ½, ½, ½. The coordinates from the E map were used as input to the LOADAT, CRYLSQ, BONOLA, and LSQPL links of the XRAY72 system. The result of the refinement is displayed in the figures. The final conventional R value ($\Sigma 1\Delta F1/1F_0 1$) is 0.048. The parameters refined were the scale factor, the secondary extinction factor, and the positional and thermal factors. The equation of a least-squares plane through C(1), C(2), C(3), and C(4) (see Fig. 2) is:

$$7.753X - 5.509Y - 4.454Z + 1.138 = 0 \qquad \text{(Eq. 1)}$$

These four atoms are all within 0.01 Å of this plane.

The phenyl ring system, C(4) + C(9), is also planar, with an average deviation of 0.004 Å. These two planes are found to be at an angle of 87.6° to one another[6].

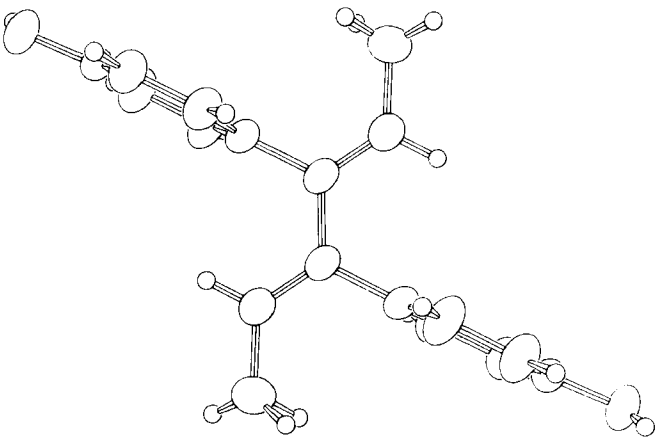


Figure 1—*The ORTEP computer plot of the whole molecule of dienestrol, showing the projection normal to the plane of C(1), C(2), C(3), and C(4).*

[2] Varian A-60 NMR spectrophotometer.
[3] Cary model 118, Applied Physics Corp., Monrovia, Calif.
[4] Picker FACS-1, Cleveland, Ohio.
[5] UNIVAC 1108.
[6] The structure factor lists were deposited with the Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204, and are available upon request to the authors.

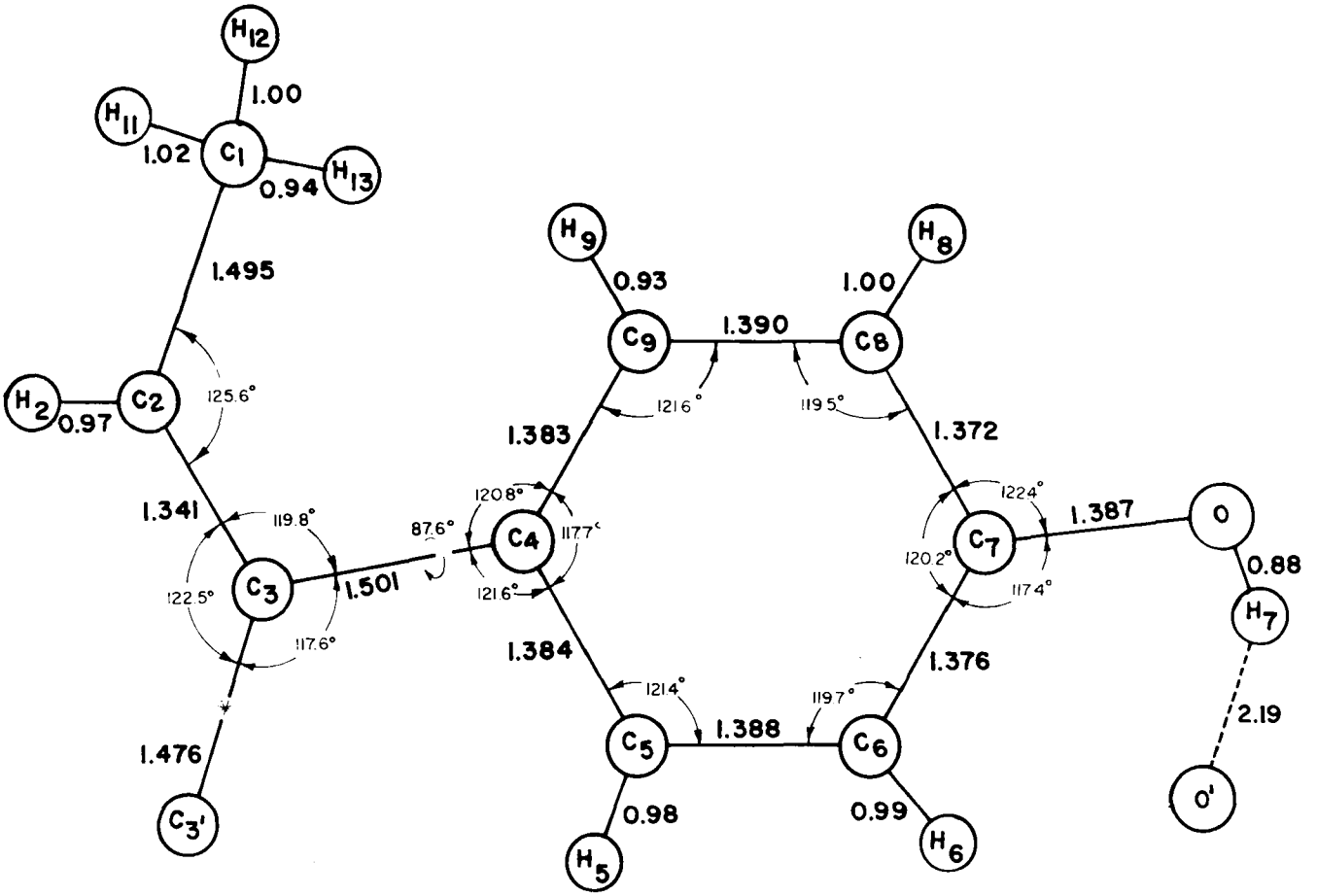


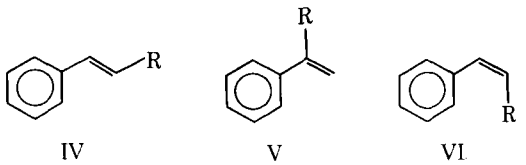
Figure 2—*Bond lengths in angstroms and angles in degrees for the refined structure of dienestrol. Since the molecule has a center of symmetry (denoted by the asterisk), only one-half of it is shown. For clarity, bond angles involving H atoms are omitted. The average estimated error in the bond angles shown is 0.15°.*

RESULTS AND DISCUSSION

The NMR spectrum of the therapeutic agent dienestrol, identical with the substance of Dodds *et al.* (1), showed that the two methyl groups have an equivalent molecular environment and identical chemical shifts, δ 1.50 ppm (doublet, J = 6.5 Hz, =CH—C*H*$_3$, 6H), and that the two vinyl protons are equivalent: δ 5.37 ppm (quartet, J = 6.5 Hz, =C*H*—CH$_3$, 2H). The only other signals in the NMR spectrum were caused by the aromatic protons at δ 6.65–7.30 ppm (multiplet, 8H) and the hydroxylic protons at δ 8.45 ppm (singlet, 2H). These findings eliminate the low symmetry Structure II as a possible dienestrol configuration.

The remaining two alternatives, I and III, could be differentiated by analysis of the UV absorption spectra of dienestrol and diacetoxy derivatives of stereoisomers I–III. Dienestrol diacetate was separately prepared from dienestrol by treatment with acetic anhydride in pyridine and recrystallization from ethanol–water, mp 113–114°. The near-UV absorption of dienestrol and its diacetate is compared to those of the other two isomeric diacetates[7] in Fig. 3. The absorption maxima of the pharmaceutical form, its diacetylated derivative, and the other diacetates are displayed at 228, 221, and 247 and 252 nm, respectively.

Evaluation of these absorption curves in terms of steric interference of substituents with normal styrene chromophore absorption allows configuration I to be assigned to dienestrol. Unsubstituted styrene, simple *trans*-β-alkylstyrenes (IV), and simple α-alkylstyrenes (V) exhibit absorption maxima in the 243–255-nm spectral region (10). On the other hand, *cis*-β-alkylstyrenes (VI) lack the characteristic styrene chromophore absorption because of out-



IV V VI

Table I—Physical Constants of Dienestrol

Molecular formula	$C_{18}H_{18}O_2$
Molecular weight	266.32 amu
Habit	Prismatic rectangular rods
Crystal size	0.25 mm along *a*, 0.4 mm along *b*, and 0.5 mm along *c*
Space group	*Pbca*
a	18.9969 (29) A
b	14.2930 (18) A
c	5.3816 (7) A
v	1461.23 A^3
ρ calculated	1.210 g/cm^3
ρ observed[a]	1.215 g/cm^3
Radiation MoKα from HOG monochromator	0.71069 A
Number of independent reflections	1457
Molecules per unit cell	4
Linear absorption coefficient, μ at MoKα	0.67 cm^{-1}
Number of parameters	128
Number of atoms in asymmetric set	19

[a] Determined by the flotation method by suspension in aqueous sodium bromide.

[7] Obtained from White Laboratories. The same source was utilized by Lane and Spialter (5).

Table II—Positional and Thermal Parameters of Atoms

				× 10^2					
	x	y	z	U_{11} or U	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O	0.22572 (6)	0.30130 (8)	0.2933 (3)	3.78 (6)	5.24 (7)	7.06 (9)	−1.31 (5)	−0.01 (6)	−0.62 (6)
C(1)	0.42099 (12)	0.65507 (14)	0.1776 (5)	5.97 (12)	5.29 (10)	8.17 (15)	0.48 (9)	−1.99 (11)	0.93 (9)
C(2)	0.47565 (9)	0.60830 (11)	0.3341 (3)	3.76 (8)	4.56 (8)	5.22 (10)	−0.28 (6)	−0.34 (7)	−0.14 (6)
C(3)	0.47118 (7)	0.52165 (10)	0.4283 (3)	3.01 (7)	4.43 (8)	3.76 (7)	−0.25 (5)	−0.08 (6)	−0.43 (6)
C(4)	0.40574 (7)	0.46486 (10)	0.3878 (3)	3.03 (7)	4.37 (7)	3.77 (8)	−0.25 (6)	−0.36 (6)	−0.03 (6)
C(5)	0.34834 (8)	0.47213 (11)	0.5451 (3)	3.65 (7)	4.78 (8)	4.92 (9)	−0.15 (6)	0.40 (6)	−1.08 (7)
C(6)	0.28820 (8)	0.41879 (11)	0.5085 (4)	3.39 (8)	5.14 (8)	5.75 (10)	−0.29 (6)	0.94 (7)	−0.59 (7)
C(7)	0.28535 (7)	0.35675 (9)	0.3132 (3)	2.99 (7)	3.87 (7)	4.98 (9)	−0.24 (5)	−0.43 (6)	0.46 (6)
C(8)	0.34105 (10)	0.34920 (13)	0.1522 (3)	4.71 (9)	6.29 (10)	4.50 (9)	−1.37 (7)	0.23 (7)	−1.56 (8)
C(9)	0.40093 (9)	0.40311 (14)	0.1910 (4)	4.00 (9)	7.49 (11)	4.36 (9)	−1.48 (8)	0.77 (7)	−1.63 (8)
H(11)	0.403	0.616 (4)	0.031 (13)	18 (2)					
H(12)	0.372 (3)	0.636 (3)	0.225 (9)	16 (1)					
H(13)	0.419 (2)	0.720 (2)	0.202 (7)	11 (1)					
H(2)	0.518 (1)	0.645 (1)	0.363 (4)	4.8 (5)					
H(5)	0.348 (1)	0.515 (1)	0.687 (4)	6.5 (6)					
H(6)	0.248 (1)	0.421 (1)	0.622 (4)	4.9 (5)					
H(7)	0.225 (2)	0.273 (2)	0.149 (6)	9.2 (9)					
H(8)	0.337 (1)	0.304 (2)	0.010 (5)	8.3 (7)					
H(9)	0.439 (1)	0.398 (2)	0.083 (5)	7.3 (6)					

isotropic temperature factors are of the form:

$$\exp[-8\pi^2 U(\sin\theta/\lambda)^2]$$

anisotropic temperature factors are of the form:

$$\exp[-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2klb^*c^*U_{23})]$$

of-plane distortions of the vinyl group with respect to the aryl π-system brought about by the steric hindrance between the alkyl substituent and the hydrogen at the *ortho*-position (11). Consequently, the λ_{max} at 221 nm displayed by dienestrol diacetate in Fig. 3 is incompatible with structural types IV or V, which leaves by elimination the *cis*-β-alkylstyrenic Structure I, analogous with VI. The fact that the other two stereoisomeric diacetates exhibit near-UV absorption consistent with the preservation of the styrene chromophore confirms their concordance with Structures II and III.

Conclusions consistent with these interpretations were also obtained from examination of scale molecular models[8] of the geometric isomers. The models suggest that the two olefinic bonds in stereoisomer I readily attain coplanarity with each other, without strain, outside the planes of the aryl nuclei, whereas prohibitive tension would be required to reach double bond conjugation in Structures II and III.

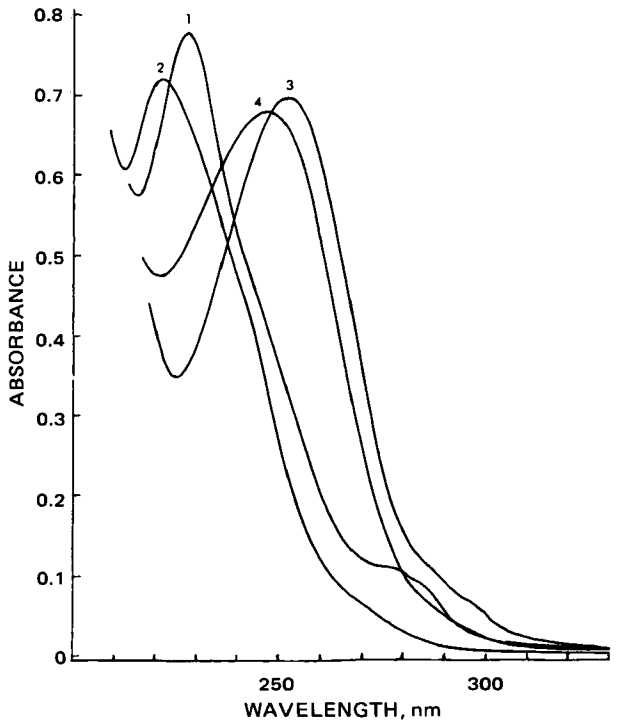


Figure 3—*Electronic absorption spectra of: (1) the active drug dienestrol, (2) the diacetate of I, (3) β-dienestrol diacetate, and (4) γ-dienestrol diacetate. The concentrations are: (1) 2.68 × 10^{-5} M (ε = 29,100), (2) 2.78 × 10^{-5} M (ε = 25,900), (3) 3.11 × 10^{-5} M (ε = 22,600), and (4) 3.50 × 10^{-5} M (ε = 19,600).*

[8] Corey–Pauling–Koltun models, Schwarz BioResearch, Orangeburg, N.Y.

The conjugation of the two vinyl groups in I offers compensatory stabilization energy for the lost styrene π-delocalization and retains a butadiene chromophore characterized by an absorption maximum at 217 nm. This may be interpreted as additional evidence for the assignment of configuration I to dienestrol, since of the four compounds in Fig. 3, only the drug and its diacetate have absorption maxima compatible with the presence of an isolated butadiene chromophore.

These deductions are in accord with the known chemical reactivity of dienestrol and its diacetate toward adduct formation with Diels–Alder dienophiles[9]. This evidence is consistent with configuration I but would not be expected with III, since planarity of the butadiene system is required for this reaction; hindrance from the methyl groups in III would prevent this planar *cis*-conformation.

Although these lines of evidence were compelling, they nevertheless were based on indirect inferences. Unambiguous configurational assignment in complete accord with the preliminary deductions was obtained by single-crystal X-ray diffraction. Table I summarizes the physical constants obtained, and Table II gives the coordinates and temperature parameters of the asymmetric atoms of the unit cell.

Figure 1 is a computer-produced view of the molecule perpendicular to the plane of the hexadiene chain, as determined by the crystallographic analysis. Assignments of bond lengths and bond angles in the crystalline state are shown in Fig. 2. The structure of dienestrol is I, in agreement with the postulation of Koch and coworkers (2, 3) but contrary to the conclusions of Lane and Spialter (5).

A least-squares plane calculated from the coordinates of C(1), C(2), C(3), C(3′), C(2′), and C(1′) shows that these atoms of the hexadiene chain are planar within 0.02 Å. In contrast, the phenyl rings lie virtually perpendicular (87.6°) to the hexadiene system and are thus out of conjugation with the remainder of the molecule. These findings for the crystalline state buttress the interpretation of the electronic spectrum of dienestrol in solution and support arguments based on molecular models and chemical reactivity.

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Stabilizing Effect of Inorganic Phosphate Salts on Antibiotic–Steroid Ophthalmic Preparations

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Abstract □ Drocinonide phosphate potassium forms an insoluble complex with neomycin sulfate in aqueous solution. Dibasic sodium phosphate can be employed in an ophthalmic formulation to prevent the formation of this precipitate without affecting the stability of the steroid or the bioactivity of the antibiotic. Other phosphate steroid salts behaved in a like manner.

Keyphrases □ Ophthalmic formulations—antibiotic–steroid preparations, stabilizing effect of inorganic phosphate salts, drocinonide phosphate potassium–neomycin sulfate complex studied □ Steroid–antibiotic ophthalmic preparations—stabilizing effect of inorganic phosphate salts □ Antibiotic–steroid ophthalmic preparations—stabilizing effect of inorganic phosphate salts □ Drocinonide phosphate potassium—complex with neomycin sulfate, prevention of precipitation by use of inorganic phosphate salts □ Inorganic salts—as stabilizers of antibiotic–steroid ophthalmic preparations

Neomycin sulfate is a polybasic antibiotic which ionizes in aqueous solution to give a positively charged species of neomycin. A wide range of anionic compounds were studied to assess their compatibility with the antibiotic (1). Approximately 50% of the high molecular weight anionic compounds tested precipitated with neomycin sulfate as an addition complex of low solubility. The stability of neomycin was investigated in several pharmaceutical preparations, and it was found that the antibiotic was relatively stable in solution over pH 2.0–9.0 (2). All products studied showed a minimum stability of 1 year at room temperature; tablets, troches, and ointment bases were stable for 2 years.

The present report describes a method for overcoming the incompatibility of neomycin sulfate with corticosteroid drugs in aqueous ophthalmic preparations. As a model, the soluble steroid chosen for investigation was drocinonide phosphate potassium[1]. To formulate this steroid with neomycin sulfate in an ophthalmic solution, the incompatibility of the two drugs must be overcome to produce a clear, particle-free solution in which the drug remains stable and active.

EXPERIMENTAL

Materials—The following were used: neomycin sulfate[2], drocinonide phosphate potassium[1], anhydrous disodium hydrogen phospate[3], anhydrous dipotassium hydrogen phosphate[3], sodium borate[3], sodium bisulfite[3], dexamethasone sodium phosphate[4], prednisolone sodium phosphate[4], polyvinylpyrrolidone[5], sodium formaldehyde sulfoxylate[6], sorbitol[7], polymyxin B sulfate[8], and thimerosal[9].

Stability Assay of Drocinonide Phosphate Potassium—A quantitative paper chromatographic method (3) was employed, using the solvent system of 1-butanol–ethanol–2 *N* ammonium hydroxide (5:1:2). Prior to the application of the sample, the paper strips were washed in alcohol and dried (4). The zones of steroid were located by use of a guide-strip spray reagent technique, and the zones on the untreated strips were eluted with water. An aliquot of the eluate was mixed with concentrated sulfuric acid, and the resulting fluorescence was measured spectrophotofluorometrically[10].

Microbiological Assay of Neomycin Sulfate—The microbiological activity of neomycin sulfate was determined by the cylinder-plate method described in USP XVIII (5).

[1] Also known as tetrahydrotriamcinolone acetonide dipotassium phosphate.
[2] Squibb, New Brunswick, N.J.
[3] Mallinckrodt, St. Louis, Mo.
[4] Merck, Rahway, N.J.
[5] Plasdone C-30, GAF Corp., New York, N.Y.
[6] Fine Organics, Lodi, N.J.
[7] Atlas, Wilmington, Del.
[8] Pfizer, Groton, Conn.
[9] Lilly, Indianapolis, Ind.
[10] Aminco-Bowman.