Note

Characterisation of some anomeric pairs of per-O-acetylated aldohexopyranosyl cyanides by laser-Raman spectroscopy*

ANTHONY T. TU**, W. KEVIN LIDDLE,

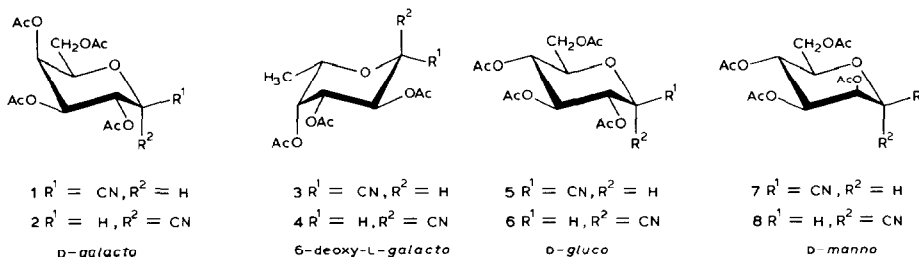*Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523 (U.S.A.)*

YUAN CHUAN LEE**, AND ROBERT W. MYERS

Department of Biology and the McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218 (U.S.A.)

Glycosyl cyanides are versatile, synthetic intermediates of considerable utility for the preparation of compounds of biological and chemical interest, including naturally occurring C-nucleosides and their analogs[1], irreversible enzyme-inhibitors[2], and substrate analogs[3], and are of interest as chiral synthons for many natural products[4]. As part of a program[5] to synthesize a series of C-glycosyl compounds that can potentially be used as "suicide substrates" and (photo)affinity-labeling reagents for glycosidases and other carbohydrate-binding proteins, compounds **1–8**, the per-O-acetylated 1,2-*trans*- and 1,2-*cis*-aldohexopyranosyl cyanides (2,6-anhydroheptononitrile peracetates) of D-galactose, L-fucose, D-glucose, and D-mannose, were recently prepared. The structure of each of these compounds was unequivocally established by elemental analysis, optical rotation, 20-MHz ^{13}C-n.m.r. and 300-MHz ^{1}H-n.m.r. spectroscopy, chemical transformation, and comparison with authentic compounds.

1 R^1 = CN, R^2 = H   3 R^1 = CN, R^2 = H   5 R^1 = CN, R^2 = H   7 R^1 = CN, R^2 = H
2 R^1 = H, R^2 = CN   4 R^1 = H, R^2 = CN   6 R^1 = H, R^2 = CN   8 R^1 = H, R^2 = CN

D-*galacto*   6-deoxy-L-*galacto*   D-*gluco*   D-*manno*

It was also of interest to characterize compounds **1–8** by vibrational spectroscopy. Unfortunately, it is well established[6] that the substitution of the alkyl group of alkylnitriles with oxygen-containing functional groups results in a decrease in the intensity of the infrared (i.r.) –C≡N stretching vibration. Moreover, this effect is known to be enhanced by increasing the number of such substituents and by attaching them to the carbon atom that bears the -C≡N group[6] In agreement with these observations, *O*-protected glycosyl cyanides do not generally exhibit the infrared –C≡N stretching-vibration[7]. Total quenching of this band in the i.r spectra of other cyano carbohydrate derivatives has been reported[8] As a result, i.r spectroscopy is often of limited value in the structural analysis of cyano carbohydrates

On the other hand, the Raman spectra of substituted-alkyl nitriles are not subject[9] to significant quenching of the -C≡N stretching vibration. Recently, Kochetkov and co-workers reported[10] that the Raman spectra of several per-*O*-acetylated 1,2-*O*-(*exo*- and *endo*-cyanoethylidene)aldohexopyranoses, as well as of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl cyanide (**1**), exhibited strong, –C≡N stretching-vibration bands in the region of 2250–2230 cm , but no corresponding bands were seen in their i.r. spectra. We have examined the per-*O*-acetylated 1,2-*trans*- and 1,2-*cis*-aldohexopyranosyl cyanides **1–8** by laser-Raman spectroscopy, and conclude that differences observed in the –C≡N and C–C stretching vibration frequencies appear to be dependent solely on the stereochemistry (axial *versus* equatorial orientation) of the anomeric cyano group

EXPERIMENTAL

The anomeric pairs of per-*O*-acetylated aldohexopyranosyl cyanides of D-galactose, L-fucose, D-glucose, and D-mannose, compounds **1–8**, were prepared by cyanation of their per-*O*-acetylated-α-D(α-L-)aldohexopyranosyl bromides with mercuric cyanide, followed by purification and crystallization[5,7b]. Raman spectra were recorded by excitation of crystalline samples with the 514.5-nm line of an argon-ion laser (Spectrophysics model SP-164) having a green interference-filter Each spectrum consists of an average of ten scans processed by a SCAMP data-acquisition unit attached to a Spex Ramalog 5 spectrophotometer. The samples were examined from 3200–200 cm^{-1} with a slit width of 200 μm and a laser power of 50–200 mW.

RESULTS AND DISCUSSION

The laser-Raman spectra of the per-*O*-acetylated aldohexopyranosyl cyanides **1–8** are reproduced in Figs. 1–4. For each of the compounds examined, a single, sharp peak was observed in the region of 2262–2242 cm^{-1}. This region corresponds to the expected frequency for the –C≡N stretching-vibration[9] Each of the per-*O*-acetylated aldohexopyranosyl cyanides previously shown by conformational analysis to have an equatorial, anomeric cyano group (the β anomers **1, 3,**

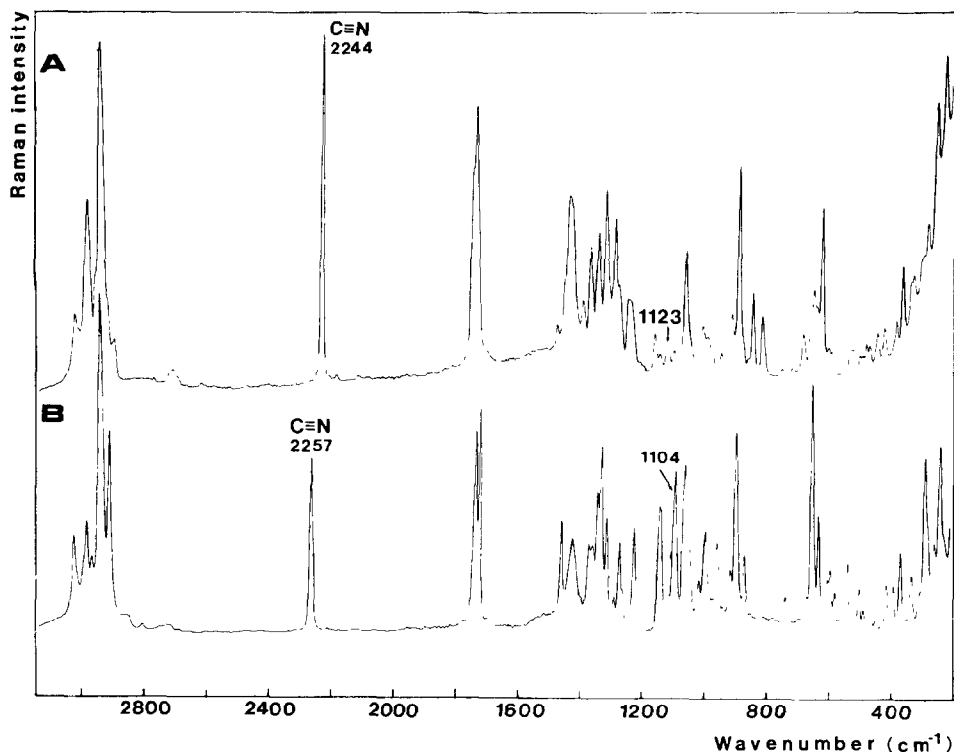Fig. 1. Laser-Raman spectra of (A) 2,3,4,6-*tetra*-*O*-acetyl-α-D-galactopyranosyl cyanide (2) and (B) its β anomer (1) in the solid phase.

5, and **7**) exhibited the –C≡N stretching-vibration in the region of 2262–2257 cm^{-1} (see Table I). Those compounds known to have an axial, anomeric cyano group (the α anomers **2, 4, 6,** and **8**) displayed lower –C≡N stretching-frequencies, from 2244–2242 cm^{-1} (see Table I).

Thus, the difference in the –C≡N stretching-vibration frequencies of the equatorial and axial anomeric cyano groups of per-*O*-acetylated aldo-hexopyranosyl cyanides is considerable. The differences are at least 12 cm^{-1} and, on the average, ~16 cm^{-1}. Moreover, these values of ν(–C≡N *eq.*) and ν(–C≡N *ax.*) are not affected by changes in the configurations of the skeletal carbon atoms, the conformation, or the substituents on C-6.

This apparently exclusive dependence of ν(–C≡N) on the equatorial or axial orientation of the cyano group of per-*O*-acetylated aldohexopyranosyl cyanides makes laser-Raman spectroscopy a valuable tool for the determination of their anomeric configuration, provided that information on the favored conformation of the pyranosyl moiety is available. Conversely, the value of ν(–C≡N) of a per-*O*-acetylated aldohexopyranosyl cyanide of known anomeric configuration should prove useful in conformational analysis. In this regard, it is of interest that an i.r.-
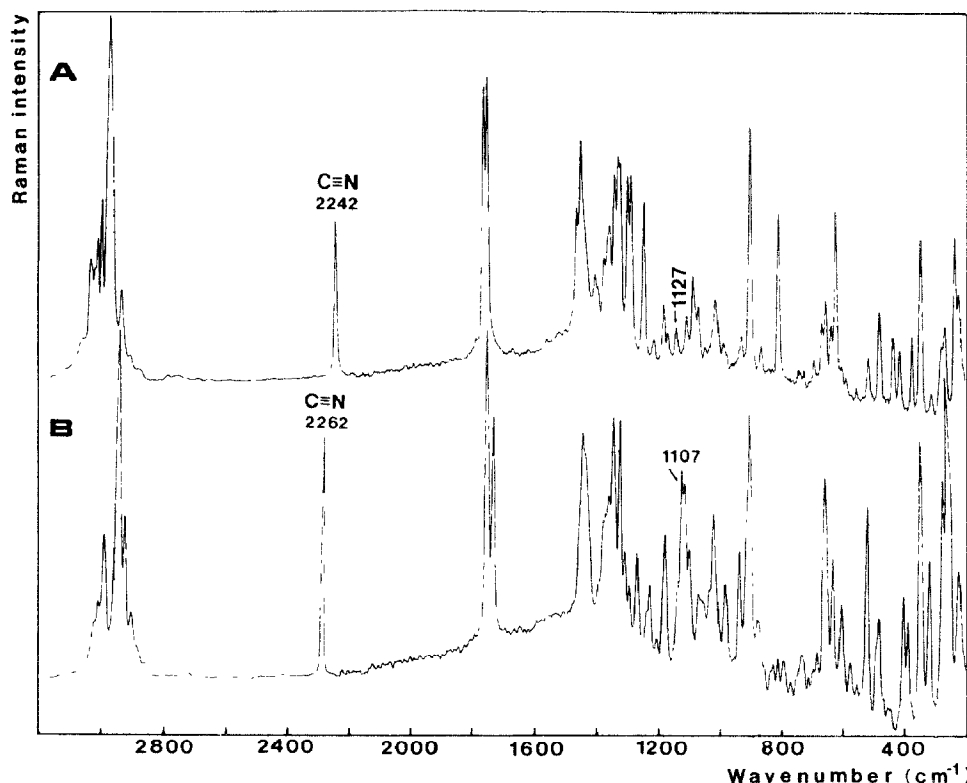
Fig. 2   Laser-Raman spectra of (A) 2,3,4-tri-O-acetyl-α-L-fucopyranosyl cyanide (4) and (B) its β anomer (3) in the solid phase

spectroscopic study of a series of anomeric pairs of glycosyl isocyanides by Descotes and co-workers[11] demonstrated a similar dependency of the -N≡C stretching-vibration frequency on the stereochemistry of the anomeric isonitrile group [ν(-N≡C eq.) 2146–2141 cm⁻¹; ν(-N≡C ax.) 2129–2123 cm⁻¹.]

It was anticipated that the stretching-vibration frequency of the C–C bond between C-1 and the cyanide carbon atom exhibited by per-O-acetylaldohexopyranosyl cyanides having equatorial and axial anomeric cyano groups should differ. Typically[12], the C–C stretching-vibration appears in the region from 1140–1000 cm⁻¹. Examination of the laser-Raman spectra of compounds 1–8 revealed the presence of a distinct band in the region from 1109–1104 cm⁻¹ for all of the compounds having an equatorial anomeric cyano group (the β anomers 1, 3, 5, and 7). With the exception of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl cyanide (6), no band was observed in this region for any of the compounds having an axial anomeric cyano group (the α anomers 2, 4, and 8). On the other hand, the α anomers 2, 4, and 8, but not 6, exhibited a band in the region of 1127–1122 cm⁻¹ that was absent from the spectra of the β anomers.

It is not possible to assign, definitely, the 1109–1104-cm⁻¹ band to the eq. C–C stretching-vibration and the 1127–1122-cm⁻¹ band to the ax. C–C bond vibration
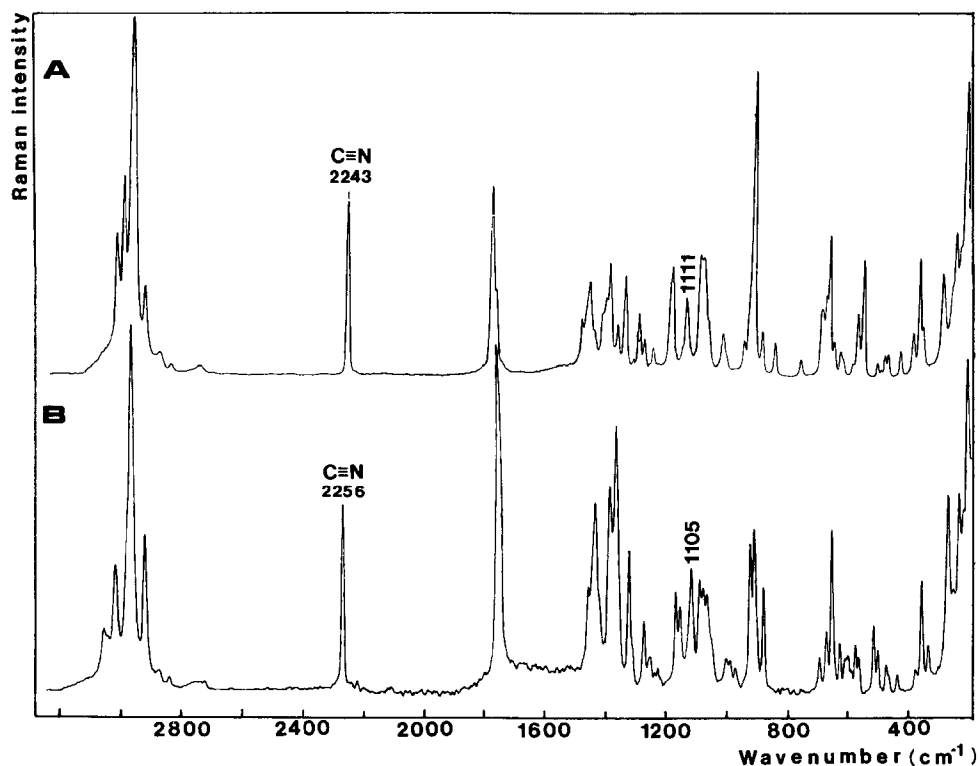
Fig. 3. Laser-Raman spectra of (A) 2,3,4,6-*tetra-O*-acetyl-α-D-glucopyranosyl cyanide (**6**) and (B) its β anomer (**5**) in the solid phase.

TABLE I

CHARACTERISTICS OF ANOMERIC C≡N AND C–C STRETCHING-VIBRATION BANDS IN THE RAMAN SPECTRA OF PER-*O*-ACETYLALDOHEXOPYRANOSYL CYANIDES (**1–8**)

Compounds	Stretching vibration (cm^{-1})		
	C≡N	C–C	
		Region I	Region II
Equatorial –C≡N (β anomers)			
1	2257[a]	1104	none
3	2262	1107	none
5	2256	1109	none
7	2259	1109	none
Axial –C≡N (α anomers)			
2	2244	none	1123
4	2242	none	1127
6	2243	none[b]	none
8	2242	none	1122

[a]Kochetkov and co-workers[10] reported ν(–C≡N) 2250 $^{-1}$ for this compound. [b]For this compound, a band was observed at 1111 cm^{-1}.
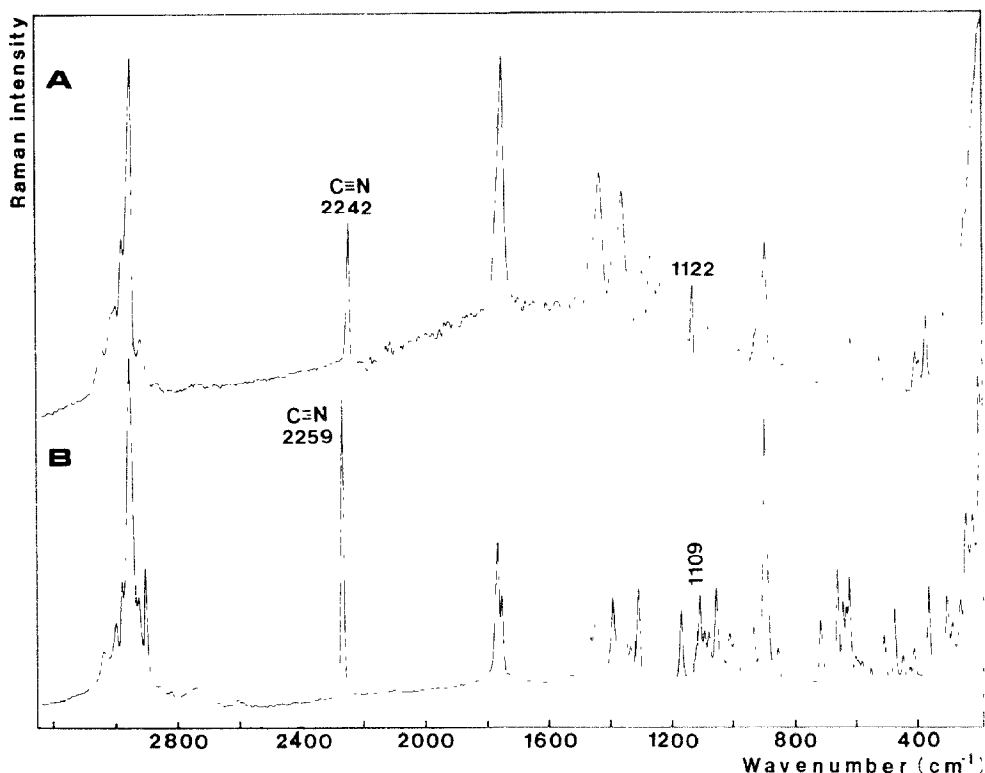
Fig. 4. Laser-Raman spectra of (A) 2,3,4,6-*tetra*-*O*-acetyl-α-D-mannopyranosyl cyanide (8) and (B) its β anomer (7) in the solid phase

without performing isotopic-substitution experiments. It does, however, seem reasonable to assume that the C–C-bond stretching-frequencies should be different for compounds respectively having axial and equatorial anomeric cyano groups, that these differences should be relatively independent of changes in the rest of the molecule, and that these differences should appear in the C–C stretching region of 1140–1000 cm⁻¹. In any event, the laser-Raman, C–C stretching-vibrations exhibited by per-*O*-acetylaldohexopyranosyl cyanides appear to be useful for confirming the stereochemical assignment of the anomeric cyano group.

In the past, i.r. spectroscopy has been one of the most important instrumental methods for the analysis of carbohydrate structure[13]. More recently, Raman spectroscopy has become[14] a powerful tool for the elucidation of carbohydrate structure, including such areas as functional-group identification, assessment of anomeric configuration, and determination of favored conformation. Raman spectroscopy has several inherent advantages over i.r. spectroscopy[15]; for example, Raman spectra can be obtained directly for solid samples (without admixture with Nujol or KBr), or for an aqueous solution of the compound of interest, and the resulting absorption peaks are frequently sharper than those in the corresponding i.r

spectra, thus providing more-precise information. An additional, important advantage of Raman spectroscopy is that certain bondings, including C–N, C=S, and S–H, yield strong Raman bands, whereas these bands are frequently weak in, or absent from, the corresponding i.r. spectra.

In the present study, these features of laser-Raman spectroscopy have been effectively utilized in order to examine the $-C\equiv N$ stretching-vibrations of per-O-acetylaldohexopyranosyl cyanides of the 1,2-*trans* and 1,2-*cis* configuration. As a result, we have been able to correlate the value of $\nu(-C\equiv N)$ with the stereochemistry of the anomeric cyano group, and suggest that laser-Raman spectroscopy may prove of value in the structural analysis of other cyano carbohydrate derivatives.

REFERENCES

1 S. HANESSIAN AND A. G. PERNET, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 111–188.
2 M. BROCKHAUS AND J. LEHMANN, *FEBS Lett.*, 62 (1976) 154–156; M. L SINNOTT AND P. J. SMITH, *Biochem. J.*, 175 (1978) 525–538; O. P. VAN DIGGELEN, H. GALJAARD, M. L. SINNOTT, AND P. J. SMITH, *ibid.*, 183 (1980) 337–343.
3 M. BROCKHAUS AND J LEHMANN, *Carbohydr. Res.*, 53 (1977) 21–31; E. J. HEHRE, C. F. BREWER, T. UCHIYAMA, P. SCHLESSELMANN, AND J LEHMANN, *Biochemistry*, 19 (1980) 3557–3564.
4 For recent reviews, see: S. HANESSIAN, *Acc. Chem. Res.*, 12 (1979) 159–165; B. FRASER-REID AND R. C. ANDERSON, *Fortschr. Chem. Org. Naturst.*, 39 (1980) 1–61, A. VASELLA, in R. SHEFFOLD (Ed.), *Modern Synthetic Methods*, Vol. 2, Verlag Sauerlander, Aarau, Switzerland, 1980.
5 R. W. MYERS AND Y. C. LEE, *Abstr. Int. Symp. Carbohydr. Chem., 11th*, Vancouver, B. C., Canada, 1982, I-30.
6 L. J. BELLAMY, *The Infra-Red Spectra of Complex Molecules*, Vol 1, Halsted Press, London, 1975, pp. 294–298.
7 For examples, see: (a) B. COXON AND H. G. FLETCHER, JR., *J. Am. Chem. Soc.*, 85 (1963) 2637–2642; (b) 86 (1964) 922–926, (c) B. COXON, *Tetrahedron*, 22 (1966) 2281–2302; (d) H. S. EL KHADEM AND E. S. H. EL ASHRY, *Carbohydr. Res.*, 32 (1974) 339–348; (e) E. M. ACTON, A N. FUJIWARA, L. GOODMAN, AND D. W. HENRY, *ibid.*, 33 (1974) 135–151; (f) T HUYNH-DINH, C. GOUYETTE, AND J. IGOLEN, *Tetrahedron Lett*, (1980) 4499–4502; (g) G. GRYNKIEWICZ AND J. N. BEMILLER, *Carbohydr. Res.*, 108 (1982) 229–235; (h) R. W. MYERS AND Y. C. LEE, unpublished results.
8 For examples, see (a) ref. 7(a); (b) V. I. BETANELI, M. V. OVCHINNIKOV, L. V. BACKINOWSKY, AND N. K. KOCHETKOV, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 28 (1979) 2561–2567; (c) J.-M. BOURGEOIS, *Helv. Chim. Acta*, 58 (1975) 363–372.
9 J. P. JESSON AND H. W. THOMPSON, *Proc. R. Soc. London, Ser. A*, 268 (1962) 68–78.
10 V. I. BETANELI, M. V. OVCHINNIKOV, L. V. BACKINOWSKY AND N.K. KOCHETKOV, ref. 8b; *Carbohydr Res.*, 68 (1979) c11–c13; V. I. BETANELI, M. M. LITVAK, L V. BACKINOWSKY, AND N.K. KOCHETKOV, *ibid.*, 94 (1981) c1–c4
11 P. BOULLANGER, D. MARMET, AND G. DESCOTES, *Tetrahedron*, 35 (1979) 163–167.
12 A. T. TU, *Raman Spectroscopy in Biology; Principles and Applications*, Wiley, New York, 1982.
13 For a review, see: R. S. TIPSON, *Natl. Bur. Stand. (U.S.) Monogr*, 110 (1968).
14 See ref. 12, pp. 234–255.