

Determination of the Anomeric Configuration of 2'-Deoxy-D-ribonucleosides by ¹H NMR and by Crystallographic Studies of a Novel 2'-Deoxy C-Nucleoside

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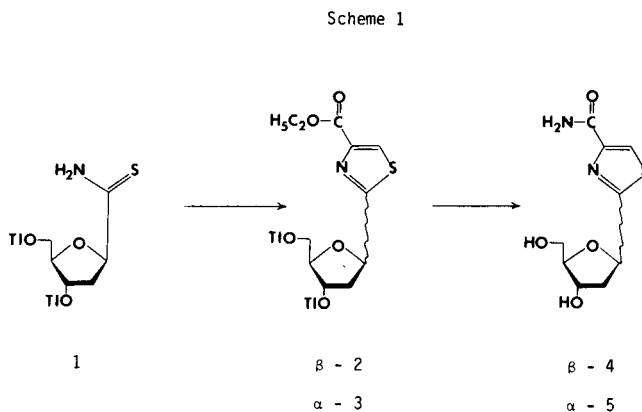
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2'-Deoxy-D-ribonucleoside analogs of biologically active 2-β-D-ribofuranosylthiazole-4-carboxamide were synthesized and the structure of the β anomer was determined by X-ray crystallography and ¹H nmr. 2'-Methylene protons of α- and β-deoxyribonucleosides were observed to exhibit characteristic patterns in the ¹H nmr which was used to distinguish between the two anomers. The method could be used to determine the anomeric configuration of both N- and C-2'-deoxyribonucleosides.

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The ¹H nmr spectra of various 2'-Deoxy-D-ribonucleosides have been studied in our laboratories and it was observed that methylene protons (H2', H2'') next to the anomeric center in α-2'-deoxy-D-ribonucleosides display more chemical shift non-equivalence than those in the corresponding β-2'-deoxy-D-ribonucleosides. Consequently the H2', H2'' resonance "band width" observed for α-2'-deoxynucleosides is generally larger than that for β-2'-deoxynucleosides. This criterion has for the first time been used to readily distinguish between the α and β configurations of deoxynucleosides, and has been found especially useful for the anomeric pairs which deviate from the "triplet-quartet-peak width" (TQPW) rule (1-3). The β-configuration determined for 2-(2'-deoxy-D-erythro-pentofuranosyl)thiazole-4-carboxamide by ¹H nmr was further confirmed by X-ray crystallography.

Recently 2-β-D-ribofuranosylthiazole-4-carboxamide synthesized in our laboratory was found to exhibit potent antitumor activity (4). Subsequently the synthesis of the corresponding 2'-deoxynucleoside, 2-(2-deoxy-β-D-erythro-pentofuranosyl)thiazole-4-carboxamide (4) was investigated. Treatment of 2,5-anhydro-3-deoxy-4,6-di-O-p-toluoyl-D-ribo-hexonothiamide (4) (1) with ethyl bromo-



pyruvate provided ethyl 2-(2-deoxy-3,5-di-O-p-toluoyl-D-erythro-pentofuranosyl)thiazole-4-carboxylates as a mixture of two anomers which were separated by silica gel column chromatography, and for convenience the anomers with fast and slow tlc mobility were denoted β (2, syrup, 52%) and α (3, syrup, 23%), respectively. Treatment of the β and α anomer with methanolic ammonia provided 2-(2-deoxy-β-D-erythro-pentofuranosyl)thiazole-4-carboxamide (4, crystals (ethanol) yield 60%, mp 144-145°) and 2-(2-deoxy-α-D-erythro-pentofuranosyl)-

Table 1

| 2'-Deoxy-D-ribonucleoside Anomer | Type | H-2', H-2'' Absorption | |
|---|-------------------------------------|---------------------------|-----------------------|
| | | Spaced Between δ (ppm) | Band Width δ (ppm) |
| 2'-Deoxy-5-azacytidine (17) | (α) Set of Separate Multiplets (SM) | 2.00-3.12 | 1.12 |
| | (β) Clustered Multiplet (CM) | 2.36-2.67 | 0.31 |
| 4-Amino-1-(2-deoxy-D-ribofuranosyl)imidazole-5-carboxamide (17) | (α) SM | 1.94-2.95 | 1.01 |
| | (β) CM | 2.20-2.63 | 0.43 |
| 2'-Deoxy-5-methyluridine (18) | (α) SM | 2.06-3.16 | 1.10 |
| | (β) CM (triplet) | 2.37-2.58 | 0.21 |
| 2'-Deoxy-5-fluorouridine (18) | (α) SM | 2.45-3.45 | 1.00 |
| | (β) CM (triplet) | 2.73-2.93 | 0.20 |
| 2-(2-Deoxy-D-ribofuranosyl)thiazole-4-carboxamide | (β) SM | 1.74-2.86 | 1.12 |
| | (β) CM | 1.89-2.52 | 0.63 |

thiazole-4-carboxamide (**5**, syrup, yield 50%), respectively (**5**) (Scheme 1).

The β configuration of **4** was confirmed by X-ray crystallography. The molecule crystallizes in space group $P2_1$ with cell dimensions $a = 7.921(2)$, $b = 6.752(2)$, $c = 10.550(3)$ Å, $\beta = 102.53(2)$ Å, and $Z = 2$. Diffraction data were obtained on a Syntex $P2_1$ diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 1.5418$ Å) with a graphite monochromator. The structure was determined by location of the sulfur atom in the Patterson map and the other atoms including all hydrogens in successive Fourier syntheses. It was refined with a block diagonal least square procedure to yield a final discrepancy R factor of 0.032 for all 1122 data with $2\theta < 140^\circ$.

The results of the determination show that the configuration at $\text{C1}'$ is β , (Figure 1) the conformation of the deoxyribose sugar ring is $\text{C2}'$ endo and the glycosidic torsion angle ($\text{O1}'\text{-C1}'\text{-C2}\text{-S}$) is 40.2° . The interbond $\text{H2}',\text{H2}''$ angle is 105° and the torsion angles $\text{H1}'\text{-C1}'\text{-C2}'\text{-H2}''$ and $\text{H1}'\text{-C1}'\text{-C2}'\text{-H2}'$ are 39° and 155° respectively. The data correlated closely with the ^1H nmr of **4** as described below.

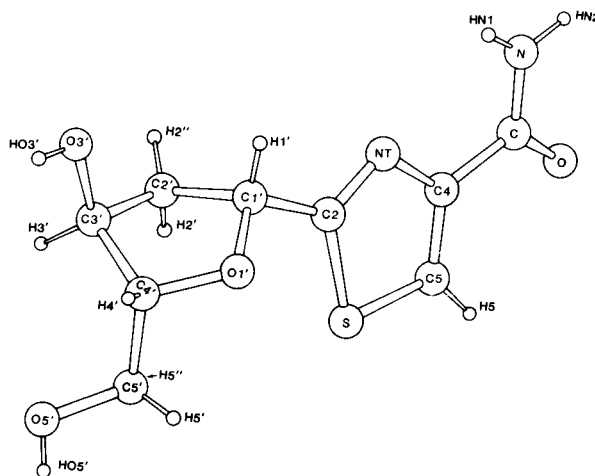


Figure 1

The formation of both the α and β anomers during the synthesis of 2'-deoxy-ribonucleosides by the direct glycosylation of an aglycone or by elaboration of the latter in a suitably functionalized sugar is not uncommon. However,

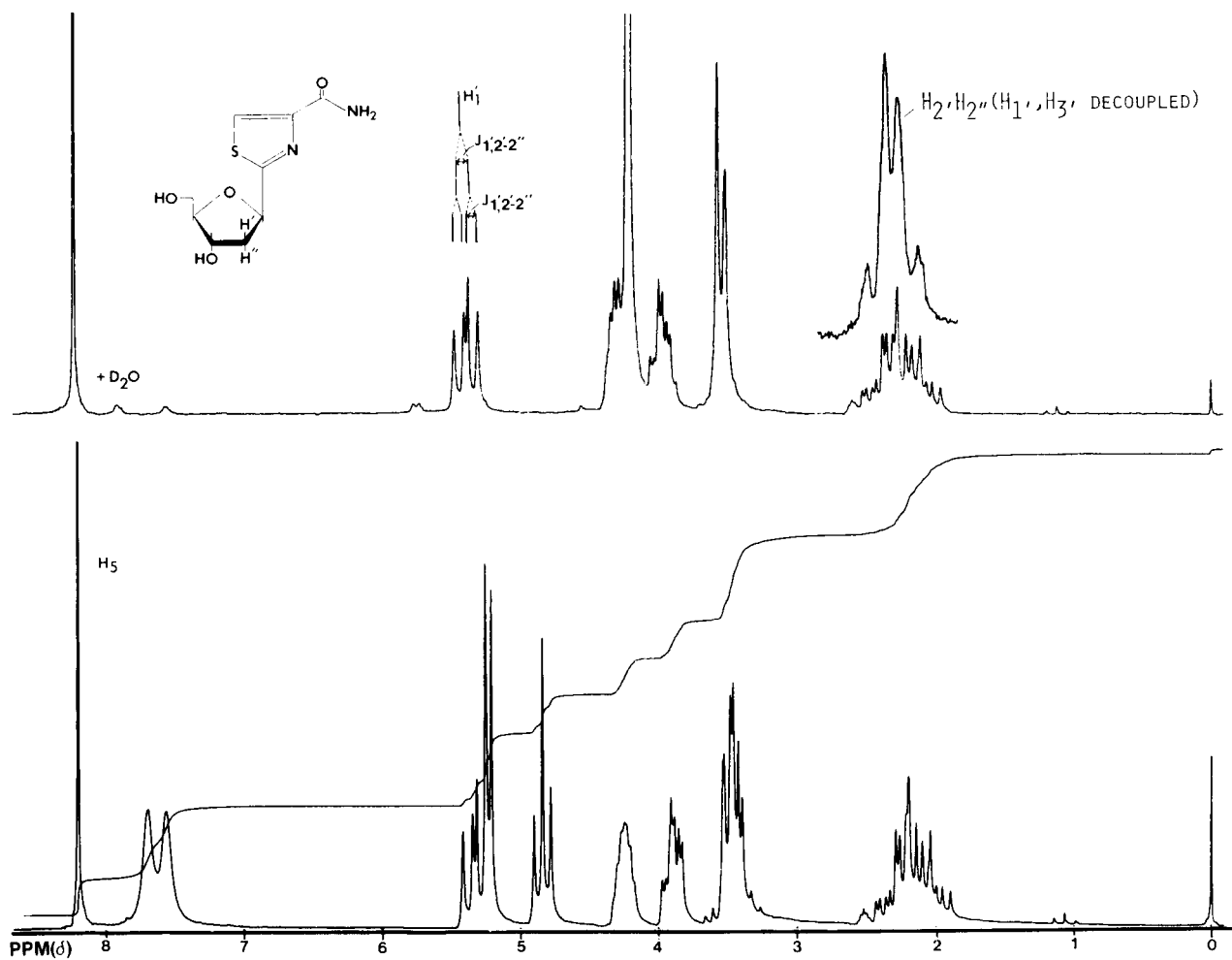


Fig. 2. ^1H FT-NMR spectra (90 MHz) in $\text{Me}_2\text{SO-d}_6$ (TMS)

the only direct method (2) available to date to readily distinguish between the α and β anomers in solution is that by ^1H nmr in which a "pseudo-triplet" with a peak width of 13.7 ± 0.5 Hz and a quartet with a peak width of 10.4 ± 0.4 Hz for the anomeric proton is indicative of β and α configurations, respectively. Based on the pioneering work by Karplus (6) and Jardetzky (1) the "triplet-peak width" rule, however, assumes that in the β -deoxynucleosides there is very little (1-3 Hz) or no difference (7) in the chemical shifts of $\text{H}2'$ and $\text{H}2''$. The deviations from this triplet-peak width rule become apparent when the difference in the chemical shift increases. Compound β -4 (Figure 2) exhibited a quartet centered at δ 5.30 \pm 0.02

$J_{1'-2',2''} = 9$ Hz and 6.2 Hz, peak width 15.2 Hz). This pattern, although a departure from the "triplet-peak width" rule, was very similar to that for α -5 (Figure 3) which also exhibited a quartet centered at δ 5.3 ($J_{1'-2',2''} = 8$ Hz and 5.2 Hz, peak width 13.2 Hz). Similar deviations from the "triplet-peak width" rule among the β deoxynucleosides have previously been reported for 2'-deoxy-5-(trifluoromethyl)uridine (8) and certain 8-substituted derivatives of 2'-deoxyadenosine (9) which also show a quartet with a peak width of 15.2 Hz for anomeric protons. Indeed, the situation in the case of 4 and 5 was more intriguing. The splitting pattern of the quartet for $\text{H}1'$ of β -4 was almost identical to that observed for the quartet for $\text{H}1'$ of α -5. Also among the α deoxynucleosides a peak width of 13.2 Hz observed for the quartet for $\text{H}1'$ of α -5 was unusual and to the best of our knowledge has never been reported before. On the basis of the data available for $\text{H}1'$ a fair assignment of the anomeric configuration of 4 and 5 by "TQPW" rule was not possible. Consequently, we focused our attention toward the methylene protons ($\text{H}2'$, $\text{H}2''$) next to the anomeric center. Certain changes in the splitting pattern of $\text{H}2'$, $\text{H}2''$ would be expected with conformational changes attributable to the difference in the steric interactions between the heterocycle and the sugar of a nucleoside. The steric interactions would be even more pronounced with the configurational changes (10) ($\alpha \leftrightarrow \beta$) in which case the difference in the splitting pattern of $\text{H}2'$, $\text{H}2''$ should become more apparent.

In the ^1H -nmr of β -4 the signal for $\text{H}2'$, $\text{H}2''$ appeared as a complex multiplet clustered at 2.20 and spaced between 1.89 to 2.52 ppm. This multiplet, after spin-spin decoupling of $\text{H}1'$ and $\text{H}3'$, was roughly resolved into an overlapping pair of doublets with $J_{2'-2''} \cong 20$ Hz which is within the range of the theoretical value (11) (19.7 Hz) for an interbond $\text{H}2'$ $\text{H}2''$ angle of 105° but the difference in the chemical shifts ($\Delta\delta \cong 12.6$) was larger than normally observed (1,7) for β -2'-deoxynucleosides. Due to small $\Delta\delta/J$ (~ 0.6) the coupling of $\text{H}2'$, $\text{H}2''$ with $\text{H}1'$ and $\text{H}3'$ would be of an ABMX system with a second order splitting pattern (7,12) and the spectrum of $\text{H}2'$, $\text{H}2''$ would be like that of a

"complex multiplet" as indeed observed in the case of β -4 (Figure 2). When $\Delta\delta$ for $\text{H}2'$, $\text{H}2''$ is very small the coupling of $\text{H}2'$, $\text{H}2''$ with $\text{H}1'$ and $\text{H}3'$ would be like that of an AA'MX system with an approximate first order splitting pattern and the spectrum of $\text{H}2'$, $\text{H}2''$ would be like that of a simple multiplet (or a triplet) as normally observed for β -2'-deoxynucleosides (13). In the ^1H -nmr of α -5 the signal for $\text{H}2'$, $\text{H}2''$ appeared as a set of three separate multiplets spread between δ 1.74 and 2.86. A multiplet accounting for one proton was centered at δ 2.62 and the other two multiplets accounting for the other one proton were spaced by 20 Hz ($J_{2'-2''}$) and centered at 2.0 (Figure 3). (Some unaccounted secondary splitting observed is due to the 3'-OH group.) A relatively large $\Delta\delta_{2'-2''}$ (56 Hz) observed for α -5 as compared to that for β -4 would be expected due to the spacing of $\text{H}2'$ (*trans* to the thiazole ring) at an "equatorial" orientation of the puckered furanose ring and the deshielding region of the thiazole ring. Consequently, $\text{H}2'$ resonates at lower field (14) as compared to $\text{H}2''$ (*trans* to $\text{H}1'$) which is shielded by the *cis* 3'-OH group and the *cis* thiazole ring (15,16). Inspection of the Corey-Pauling-Koltun model of α -5 shows that in a "locked" glycosidic conformation $\text{H}2''$ is located directly below the shielding plane of the thiazole ring. It also appears that due to the relatively large "S" atom the rotation around the glycosidic bond is restricted and a conformation close to that in the locked glycosidic position may be predominant even when in solution and on the nmr time scale. The shielding behavior of $\text{H}2''$ on one hand and that deshielding of $\text{H}2'$ on the other in α deoxynucleosides is responsible for extended absorption "band width" of $\text{H}2'$, $\text{H}2''$ as compared to that for the corresponding β anomers. Visual examination of the Dreiding model of α -5 shows that both $\text{H}1'$ and $\text{H}3'$ have very small dihedral angles ($\sim 5^\circ$) with respect to the *cis* oriented $\text{H}2'$ whereas those of $\sim 130^\circ$ with respect to the *trans* oriented $\text{H}2''$. According to the relationship of the coupling constants with the dihedral angles (1,13), $\text{H}2'$ which would be expected to strongly couple with $\text{H}1'$ and $\text{H}3'$ (observed $J_{2'-1',3'} = 8$ Hz) appears as a multiplet whereas $\text{H}2''$ which is weakly coupled with $\text{H}1'$ and $\text{H}3'$ (observed $J_{2'-1',3'} = 5.2$ Hz) appears as a pair of multiplets. Due to the relatively large $\Delta\delta$ (56 Hz) and $\Delta\delta/J$ (2.8) for $\text{H}2'$, $\text{H}2''$, the coupling of these protons with $\text{H}1'$ and $\text{H}3'$ in case of α -5 would be similar to that of an AMXY system with an approximate first order splitting pattern as seen in Figure 3.

We have examined the ^1H nmr of several anomeric pairs of 2'-deoxy-*N*-nucleosides (Table 1) and observed in each case that the absorption of $\text{H}2'$, $\text{H}2''$ for α anomers extends to both higher and lower fields, giving a larger absorption "band width" and $\Delta\delta$ as compared to the corresponding β anomers. Consequently, the signal for $\text{H}2'$,

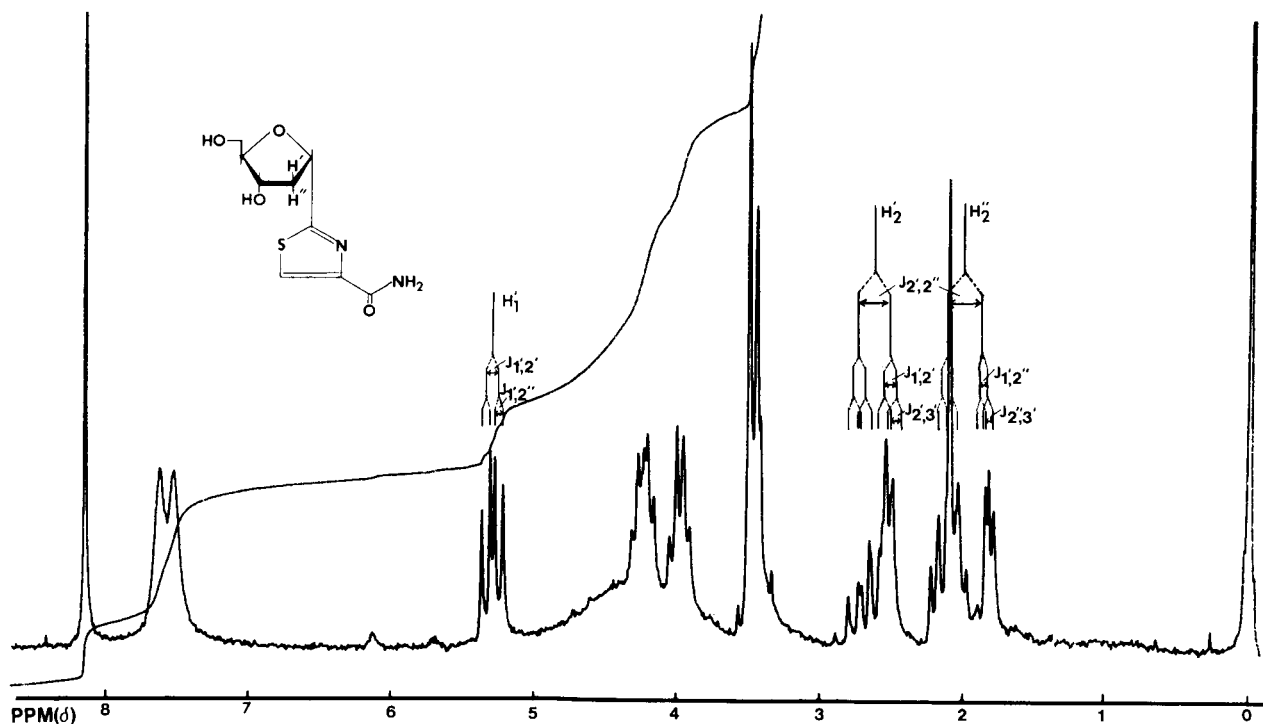


Fig. 3. ^1H FT-NMR spectra (90 MHz) in $\text{Me}_2\text{SO}-d_6$ (TMS)

$\text{H}2''$ for α anomers appears as a set of three separate multiplets (or peaks) as compared to a clustered multiplet (or triplet) observed for the same protons in the corresponding β anomers. The values for some anomeric pairs are given in Table 1 (and correlate well with β and α assignments of **4** and **5** respectively). These characteristic ^1H nmr properties of methylene protons should prove helpful in distinguishing between the α and β anomers of 2'-deoxynucleosides especially when the "TQPW" rule fails to differentiate the two anomers.

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REFERENCES AND NOTES

- (1) C. D. Jardetzky, *J. Am. Chem. Soc.*, **83**, 2919 (1961).
- (2) M. J. Robins and R. K. Robins, *ibid.*, **87**, 4934 (1965).
- (3) L. B. Townsend, in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2, W. W. Zorbach and R. S. Tipson, eds., John Wiley and Sons, New York, 1973, p. 337.
- (4) P. C. Srivastava, M. V. Pickering, L. B. Allen, D. G. Streeter, M. T. Campbell, J. T. Witkowski, R. W. Sidwell and R. K. Robins, *J. Med. Chem.*, **20**, 256 (1977); R. K. Robins, P. C. Srivastava, V. L. Narayanan, J. Plowman and K. D. Paull, *ibid.*, in press.
- (5) All compounds gave satisfactory elemental analysis.
- (6) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
- (7) R. E. Richards and T. P. Schaefer, *J. Mol. Phys.*, **1**, 331 (1958).
- (8) K. J. Ryan, E. M. Acton and L. Goodman, *J. Org. Chem.*, **31**, 1181 (1966).
- (9) R. A. Long, R. K. Robins and L. B. Townsend, *ibid.*, **32**, 2751 (1967).
- (10) M. Sundaralingam, *J. Am. Chem. Soc.*, **93**, 6644 (1971).
- (11) H. S. Gutowski, M. Karplus and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).
- (12) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance", McGraw-Hill, New York, 1959. Also R. M. Silverstein, G. C. Bassler and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, John Wiley and Sons, New York, 1974 (3rd edition), p. 159.
- (13) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).
- (14) B. Fraser-Reid and B. Radatus, *J. Am. Chem. Soc.*, **93**, 6342 (1971).
- (15) K. N. Fang, N. S. Kondo, P. S. Miller and P. O. P. Ts'o, *ibid.*, **93**, 6647 (1971).
- (16) K. N. Slessor and A. S. Tracey, *Carbohydr. Res.*, **27**, 407 (1973).
- (17) P. C. Srivastava and R. K. Robins, unpublished observations; see also reference 3, pp. 300 and 363.
- (18) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).