Determination of the absolute configuration of (+)-neopentyl-1-d alcohol by neutron and x-ray diffraction analysis

(chiral methylene group/deuterated neopentanol/alcohol dehydrogenase)

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Communicated by George A. Olah, September 6, 1994

ABSTRACT   The absolute configuration of (+)-neopentyl-1-d alcohol, prepared by the reduction of 2,2-dimethylpropanal-1-d by actively fermenting yeast, has been determined to be S by neutron diffraction. The neutron study was carried out on the phthalate half ester of neopentyl-1-d alcohol, crystallized as its strychnine salt. The absolute configuration of the (−)-strychninium cation was first determined by an x-ray anomalous dispersion study of its iodide salt. The chiral skeleton of strychnine then served as a reference from which the absolute configuration of the -O-CHD-C(CH₃)₃ group of neopentyl phthalate was determined. Difference Fourier maps calculated from the neutron data showed unambiguously that the -O-CHD-C(CH₃)₃ groups of both independent molecules in the unit cell had the S configuration. This work proves conclusively that the yeast system reduces aldehydes by delivering hydrogen to the re face of the carbonyl group. Crystallographic details: (−)-strychninium (+)-neopentyl-1-d phthalate, space group $P2_1$ (monoclinic), $a = 18.564(6)$ Å, $b = 7.713(2)$ Å, $c = 23.361(8)$ Å, $\beta = 94.18(4)°$, $V = 3336.0(5)$ Å³, $Z = 2$ ($T = 100$ K). Final agreement factors are $R(F) = 0.073$ for 2768 reflections collected at room temperature (x-ray analysis) and $R(F) = 0.144$ for 960 reflections collected at 100 K (neutron analysis).

The enzymatic reduction of an aldehyde to an alcohol is an important example of a stereospecific biological process that has been studied extensively (1). It is a prototype reaction for many biochemically significant transformations involving the coenzyme NADH. In addition, optically active deuterated alcohols of the type R-CHD-OH have been of great importance in the elucidation of the stereochemistry of displacement reactions at primary carbons (1).

The stereospecificity of enzymatic acetaldehyde reduction and ethanol oxidation was established (2–4) when it was discovered that reduction of acetaldehyde-1-d with NADH and yeast alcohol dehydrogenase (ADH) gave ethanol-1-d, which, upon enzymatic reoxidation, returned only acetaldehyde-1-d without loss of deuterium (Scheme I):
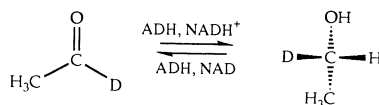
Scheme I

Simon and coworkers (5) have extended this work by using purified enzyme systems to include propyl-1-d and butyl-1-d alcohols; while Mosher and coworkers (6–8) by using the actively fermenting yeast method have produced propyl-1-d, butyl-1-d, neopentyl-1-d, and benzyl-1-d alcohols. Where the same substrates have been tested in both systems, stereo-

chemistries were identical. Although neopentyl-1-d and benzyl-1-d alcohols have not been produced by reductions using purified NADH and yeast ADH, it is firmly believed that the NADH/yeast ADH system is responsible for those reductions in the actively fermenting yeast system. The structure of horse liver ADH, which is closely related to yeast ADH, has been determined to 2.4-Å resolution by x-ray diffraction techniques (9–14). A model of the active site has been proposed in which the oxygen atom of the substrate binds directly to the zinc atom, which fixes the orientation of the substrate with respect to the coenzyme and controls the stereospecificity of the reaction (14). In this mechanism, direct hydrogen transfer from NADH to substrate is believed to take place.

For the most part, the absolute configurations of deuterated alcohols (R-CHD-OH) have been determined by comparison of NMR and polarimetry data with related molecules of known stereochemistry (15, 16). For example, the absolute configuration of ethanol-1-d has been deduced by correlation with a pyranose sugar whose configuration at the CHD center was established by NMR (17) and by correlation with 2-butanol-2-d (18) and pentane-2-d (19) of known configuration. In addition, the absolute configuration of glycolic-d acid, determined by neutron diffraction analysis (20), has been used to deduce the absolute configuration of ethanol-1-d (21). Finally, NMR spectral studies of deuterated alcohols in the presence of chiral lanthanide shift reagents (22) and mechanistic studies of asymmetric reactions involving optically active Grignard reagents (19) have given additional information regarding the absolute configuration of deuterated alcohols.

In all of the studies mentioned above, with the exception of that on glycolic acid (20), absolute configuration assignments were accomplished by indirect spectral comparisons. It is important to confirm these results by neutron diffraction, which can locate the positions of H and D atoms directly (23). In this paper we describe the results of such a study.

The target molecule in this work is (+)-neopentyl-1-d alcohol (I), obtained via the reduction of 2,2-dimethylpropanal-1-d by actively fermenting yeast (8). The optical rotation of this compound (α_D^{20}) was originally reported to be zero in acetone solvent but was subsequently determined to be (+) in hexane with a plane-positive optical rotatory dispersion curve (see footnote 35 and figure 1 of ref. 7). For the purpose of the neutron study, it was necessary to incorporate the alcohol with a chiral reference molecule. The alcohol was converted into its corresponding phthalate half ester (II), which was then used to form salts with chiral cations. A large variety of different chiral alkaloids were screened, including

quinine, quinidine, cinchonine, cinchonidine, brucine, and strychnine. The strychnine salt (**III**) of neopentyl phthalate gave the best crystals, and the overall reactions are shown in Scheme **II**.

Scheme **II**

FIG. 2. Absolute configuration of strychnine determined by Peerdeman (27).

ture (Fig. 2), we have redetermined the absolute configuration of (−)-strychnine by the anomalous dispersion method on its hydroiodide salt.

EXPERIMENTAL SECTION

Crystallization of (−)-Strychnine Hydroiodide. Crystals of (−)-strychnine hydroiodide were grown from an ≈1:1 aqueous mixture of strychnine and HI with a slight excess of HI. (−)-Strychnine was dissolved in water to a concentration of 0.4 M, and then a stoichiometric amount of hydroiodic acid was added into the heated water solution (≈80°C). The well-mixed solution was allowed to cool slowly and beautiful needle-like crystals were obtained after ≈6 h.

X-Ray Diffraction Analysis of (−)-Strychnine Hydroiodide. A crystal of dimensions 0.3 × 0.4 × 0.5 mm was chosen for the analysis. Unit-cell constants and other crystallographic details are given in Table 1. Intensities were measured on a Siemens/Nicolet $P2_1$ diffractometer utilizing graphite-monochromated MoKα radiation and the ω-scanning technique at room temperature. Two Friedel-related quadrants $[+h, +k, \pm\ell)$ and $(-h, -k, \pm\ell)]$ were collected for the determination of the absolute configuration of the molecule. Two reflections were monitored at 50-reflection intervals throughout the data collection and showed no significant changes in their intensities. An empirical absorption correction (37) was applied, as well as corrections for Lorentz-polarization effects. After data reduction, 2825 reflections having F^2 values $>3\sigma(F^2)$ were used in the structure analysis.

Previous structure determinations on the absolute configuration of (−)-strychnine had produced conflicting results. The x-ray structure analysis was originally performed by Bokhoven *et al.* (24) and by Robertson and Beevers (25, 26). The former group (24) used the isomorphous replacement method with strychninium sulfate and strychninium selenate, whereas the latter group used strychnine hydrobromide dihydrate. Both groups concluded that the absolute configuration of (−)-strychnine is that shown in Fig. 1. However, this conclusion was revised by Peerdeman (27), who recalculated the structure factors for strychnine hydrobromide and proposed an absolute configuration of (−)-strychnine (Fig. 2), which is opposite to that assigned earlier. Other structural papers (28–36) published since then do not address the absolute configuration of the strychnine skeleton. Because the absolute configuration of the strychninium cation is pivotal in this study, even though an interconnecting network of chemical evidence (35, 36) supports the Peerdeman struc-
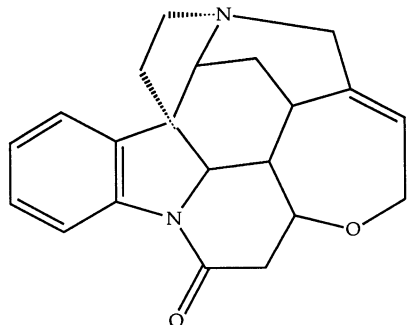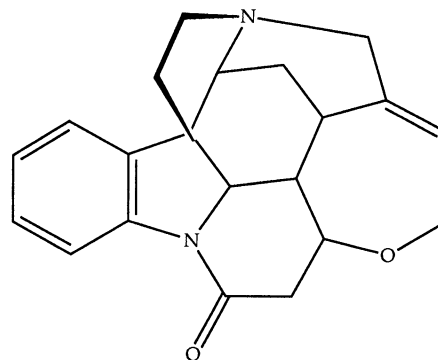
Table 1. Summary of crystal data and refinement results for strychnine hydroiodide

Parameter	Value
Space group	$P2_1$
a, Å	7.805 (2)
b, Å	7.667 (2)
c, Å	16.290 (5)
β, degrees	92.46 (2)
V, Å^3	973.8 (5)
Calculated density, g/cm^3	1.58
Crystal dimensions, mm	0.3 × 0.4 × 0.5
Wavelength, Å	0.71069
Sin θ/λ limit, Å^{-1}	0.5385
Total no. of reflections measured	2988
No. of independent reflections	2957
No. of reflections used in structure analysis	2825
No. of variable parameters	235
Final agreement factors	$R(F) = 0.067$
	$R(wF) = 0.074$

Numbers in parentheses are the estimated standard deviations of the least significant digits.

FIG. 1. Absolute configuration of strychnine determined by Bokhoven *et al.* (24).

The iodine atom was located from a Patterson synthesis. Subsequent difference Fourier maps revealed the positions of remaining nonhydrogen atoms, to yield an initial model corresponding to the absolute configuration shown in Fig. 2. Full-matrix least-squares refinement with anisotropic thermal parameters for all atoms resulted in an agreement factor of $R(wF)$ = 0.0778. Refinement of the mirror image of the molecule as shown in Fig. 1 resulted in a significantly higher R value (Rw = 0.0825). This difference in R values indicates (38) that the initial model, corresponding to the Peerdeman structure (Fig. 2), had the proper absolute configuration (27, 36). Inclusion of calculated H positions and continued least-squares refinement resulted in final agreement factors of $R(F)$ = 0.067 and $R(wF)$ = 0.074 for 2825 reflections (see *Appendix*).

Preparation of (+)-Neopentyl-1-*d* Alcohol (I) and (−)-Neopentyl-1-*d* Phthalate (II). The sample of (+)-neopentyl-1-*d* alcohol (I), m.p. 54°C, α_D^{20} +0.031 ± 0.005° (c = 30, cyclohexane) was made by the actively fermenting yeast reduction of 2,2-dimethylpropanal-1-*d* as described (8). This was converted to (−)-neopentyl-1-*d* acid phthalate (II), m.p. 68°C, α_D^{20} −0.95 ± 0.02° (c = 15, acetone) by treating the above (+)-alcohol with phthalic anhydride in pyridine as described (8); note that the (+)-alcohol gives the (−)-acid phthalate. NMR measurements showed that **II** was 82 ± 2% deuterated and the optical rotation showed that the deuterated molecules were enantiomerically pure.

Crystallization of Strychninium Neopentyl Phthalate·2CHCl₃ (III). A 1:1 molar mixture of (−)-strychnine and neopentyl phthalate (**II**) was dissolved in chloroform (≈0.5 M), and the filtered solution was evaporated to half its original volume. The resulting white precipitate (**III**) was collected, washed, and dried. It was then dissolved in a 4:1 (vol/vol) mixture of chloroform and ethyl acetate (0.1 M), and then filtered. From slow evaporation, large plate-like crystals of the chloroform solvate appeared after 2.5 days. An NMR spectrum confirmed the presence of both strychnine and neopentyl phthalate.

X-Ray Data Collection and Structure Analysis of Unlabeled Strychninium Neopentyl Phthalate·2CHCl₃ (III). A small cube-like crystal of dimensions 0.20 × 0.29 × 0.30 mm was used for the x-ray analysis. Intensities were measured with MoKα radiation. Unit-cell parameters, derived from the angular settings of 15 well-centered reflections, are presented in Table 2 along with other relevant crystal details. The space group was found to be $P2_1$ (monoclinic). One quadrant of data was collected by the $\theta/2\theta$ scan technique up to a 2θ limit of

50°. As a check on the stability of the diffractometer and the crystal, two reflections were measured at 50-reflection intervals during data collection and showed no significant variation in their intensities. During the subsequent data processing, reflections whose observed intensities were <3 times their standard deviations were discarded. The intensity data were corrected for Lorentz polarization and empirically for absorption effects (37).

Strychninium neopentyl phthalate crystallizes with two independent cations and two independent anions in the unit cell. Numerous attempts using MULTAN (39) proved to be fruitless. The structure was eventually solved using PATSEE (40), a fragment search program that utilizes integrated Patterson and direct methods. A known strychnine skeleton (28) was input into this program and subjected to a rotational and translational search procedure, to yield its correct orientation and location. Application of the program DIRDIF (41) subsequently expanded the list of known atoms and eventually revealed the positions of the second strychninium cation and the two neopentyl phthalate anions in the structure. With calculated hydrogen positions, the final anisotropic least-squares refinement cycles gave agreement factors of $R(F)$ = 0.073 and $R(wF)$ = 0.069 for 2768 reflections. The molecular plot of strychnine, given in Fig. 3, has the same configuration as that of strychnine hydroiodide determined earlier (see Fig. 2) (27).

Neutron Data Collection and Structure Analysis for Strychninium (+)-Neopentyl-1-*d* Phthalate·2CHCl₃ (III). A transparent clear plate-like crystal with approximate dimensions 2.0 × 4.9 × 0.8 mm was mounted with an adhesive on an aluminum pin oriented approximately along the b* direction and placed in a specially adapted closed-cycle refrigerator (Air Products and Chemicals, Allentown, PA, Displex model CS-202). Neutron diffraction data were collected at 100 K on an automated four-circle diffractometer (42, 43) at the Brookhaven High Flux Beam Reactor using a Ge(2 2 0) monochromated neutron beam of wavelength 1.15882(7) Å (based on KBr, a_0 = 6.6000 Å at 298 K). One reflection (2 0 0) was monitored during cooling (298 K to 100 K) to check the condition of the crystal. Unit-cell constants, determined from the averaged 2θ values of 16 Friedel pairs, and other experimental details are given in Table 2.

Intensities of Bragg reflections were measured by means of $\theta/2\theta$ step scans, with data being accumulated at each step for a predetermined monitor count of the incident beam. The collection of low-angle (5° <2θ <60°) data was completed

Table 2. Summary of crystal data and refinement results for strychninium neopentyl phthalate

Parameter	Value	
	X-ray at room temp (nondeuterated sample)	Neutron at 100 K (deuterated sample)
Space group	$P2_1$	$P2_1$
a, Å	18.559 (29)	18.564 (6)
b, Å	7.871 (8)	7.713 (2)
c, Å	23.913 (23)	23.361 (8)
β, degrees	95.67 (10)	94.18 (4)
V, Å³	3476.5 (72)	3336.0 (5)
Calculated density, g/cm³	1.09	1.14
Crystal dimensions, mm	0.20 × 0.28 × 0.30	2.0 × 4.9 × 0.8
Wavelength, Å	0.71069	1.15882 (7)
Sin θ/λ limit, Å⁻¹	0.544	0.685
Resolution, Å	1.84	1.46
Total no. of reflections measured	5582	6249
No. of independent reflections	5553	1690
No. of reflections used in structure analysis	2768	960
No. of variable parameters	829	1308
Final agreement factors	$R(F)$ = 0.073	$R(F)$ = 0.144
	$R(wF)$ = 0.069	$R(wF)$ = 0.150

Numbers in parentheses are as in Table 1.

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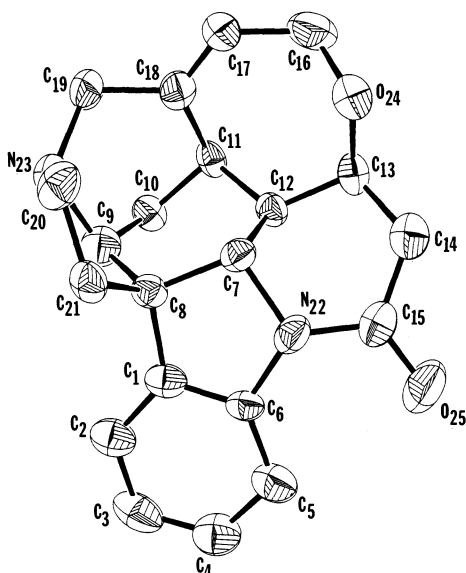*Proc. Natl. Acad. Sci. USA 91 (1994)* 12875

FIG. 3. Absolute configuration of the strychnine cation from the x-ray results for strychnine hydroiodide.

with a fixed scan of 3.0° and 75 steps per scan. For the high-angle (60° < 2θ < 105°) data, a variable scan was used with width ranging from 3.0° for 2θ = 60° to 5.18° for 2θ = 105° and step size adjusted to yield between 60 and 90 steps per scan. Three intense reflections [(1 1 7), (−2 5 −4), (0 −5, −5)] were monitored at 200-reflection intervals and no significant variations in the intensities were observed.

The raw intensities were integrated and background corrections were applied based on the counts of approximately seven steps on either end of each scan. The squared observed structure factor (F_{obs}^2) and standard deviation (σ) for each reflection were obtained upon applying Lorentz and absorption corrections (44). The latter were calculated by numerical integration over a Gaussian grid of 64 sampling points. Values for the transmission coefficients ranged from 0.621 to 0.833. The validity of the absorption correction was confirmed by comparison with data from an azimuthal intensity scan for reflection (0 −6 0). Averaging of equivalent reflections yielded 1690 independent reflections with a weighted agreement factor of wR_{int} = 0.056 and an unweighted agreement factor of R_{int} = 0.076.

The space group was determined to be $P2_1$ in the low-temperature neutron case, consistent with the room-temperature x-ray data. Initial coordinates for the nonhydrogen atoms were taken from the x-ray results. The hydrogen
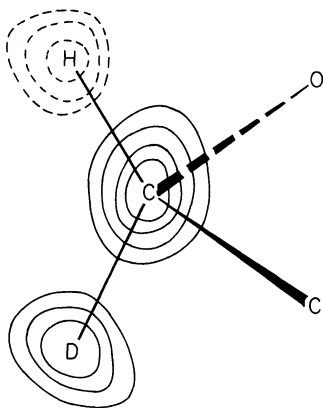

FIG. 4. Difference scattering density map in the plane passing through C_{60}, H_{60}, and D_{60} [from the neutron analysis of strychninium (+)-neopentyl phthalate]. Solid contours are positive; broken contours are negative.
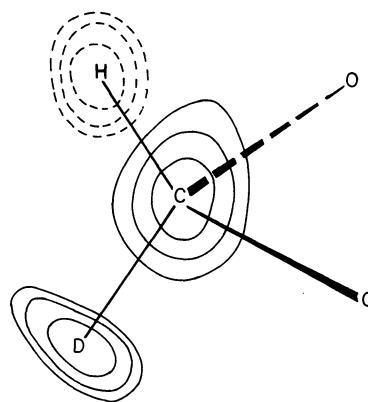

FIG. 5. Difference scattering density map in the plane passing through C_{77}, H_{77}, and D_{77} [from the neutron analysis of strychninium (+)-neopentyl phthalate]. Solid contours are positive; broken contours are negative.

positions were recalculated each cycle after the refinement of nonhydrogen atoms but the hydrogen atoms were refined with their own free temperature factors. In this way, the number of variable parameters was reduced. The structure was refined using a modified version of SHELX76 (45), which can accept more than the usual number of atoms. Since only 960 reflections remained after the 3σ cut-off, a blocked-refinement technique was utilized in which each of the four ions were refined separately, with no more than 350 variables being refined in any given cycle. The final cycles of refinement, with all the atoms assigned anisotropic thermal parameters, gave agreement factors of $R(F)$ = 0.144 and $R(wF)$ = 0.149 for 960 reflections (see *Appendix*). The rather poor agreement factor (a result of the small size of the crystal used in data collection) is not of concern here: We are mainly interested in obtaining the absolute configuration of the anions, rather than in obtaining accurate distances and angles.

To determine the absolute configuration of the CHDRR' group, difference Fourier maps were calculated (46) in the planes of the two crystallographically independent CHD groups in the structure. They each reveal two positive peaks and one negative peak, as shown in Figs. 4 and 5. The two positive peaks correspond to the carbon and deuterium atoms, whose scattering lengths are 6.648 fm and 6.70 fm, respectively; while the negative peak is the hydrogen atom that has a scattering length of −3.74 fm. These positions show unambiguously that both neopentyl phthalate anions have the *S* configuration (Fig. 6).

Because the shape of the crystal is rather thin and the volume of the crystal is low, particularly considering the number of atoms to be found, high agreement factors were
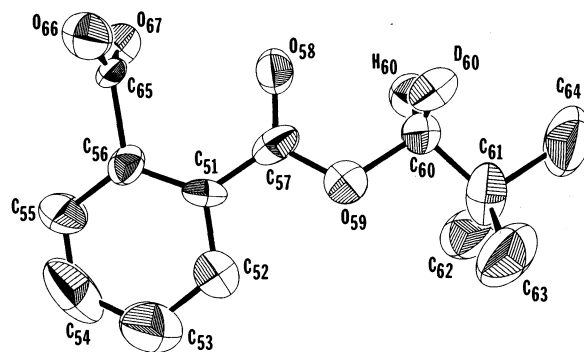

FIG. 6. Molecular plot of one of the (*S*)-neopentyl-1-*d* phthalate anions. The positions of the D and H atoms are revealed by the difference scattering density maps calculated from the neutron data (Figs. 4 and 5).
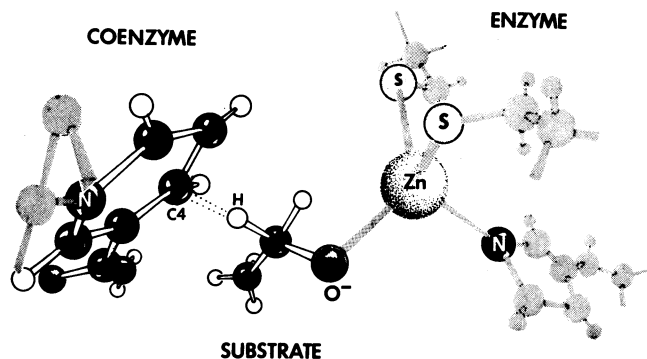
FIG. 7. Schematic diagram illustrating the active site of the enzyme horse liver ADH. Reproduced with permission from ref. 14 (copyright, Wiley-Interscience).

unavoidable. Nevertheless, the positions of the hydrogen and deuterium atoms were determined unambiguously. The peaks of C, H, and D (for both independent anions in the unit cell) are clearly seen in the difference Fourier maps (Figs. 4 and 5). Both atoms C60 and C77 have one positive peak (D) and one negative peak (H) near them, with distances and angles consistent with a tetrahedrally bonded carbon atom (average parameters: C–H = 1.05 Å, C–D = 1.19 Å, and H–C–D = 104°). The intensities of the deuterium peaks, obtained from the refinement of the neutron data, correspond to 83% deuteration, although the error in this determination may be somewhat high. As shown in the ORTEP (47) plot (Fig. 6), the absolute configuration of the neopentyl-1-*d* alcohol is *S*, as originally predicted (1, 8).

CONCLUSION

This neutron diffraction analysis unambiguously confirms that the stereochemistry of the enzymatic aldehyde/alcohol transformation suggested by other investigators is correct: In other words, the A class of enzymes (48) (including yeast and liver ADH) transfers a hydrogen atom from the *re* face of the dihydronicotinamide ring of NADH to the *re* face at the carbonyl carbon atom of 1-deuterioaldehydes (49) (Scheme I), to give *S* 1-deuterioalcohols (Fig. 7).

APPENDIX

Table	Title of supplementary table
S1	Final atomic coordinates for strychnine hydroiodide (x-ray study)
S2	Final atomic coordinates for strychninium neopentyl phthalate (x-ray study)
S3	Final atomic coordinates for strychninium neopentyl phthalate (neutron study)
S4	Anisotropic temperature factors for strychnine hydroiodide (x-ray study)
S5	Anisotropic temperature factors for strychninium neopentyl phthalate (x-ray study)
S6	Anisotropic temperature factors for strychninium neopentyl-1-*d* phthalate (neutron study)
S7	Bond distances (Å) and angles (degrees) for strychnine hydroiodide (x-ray study)
S8	Bond distances (Å) and angles (degrees) for strychninium neopentyl phthalate (x-ray study)

These supplementary tables are available from one of the authors (R.B.).

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