intracellular distribution of the substance. It seems unlikely that reserpine which is very sparingly soluble in water at physiological pH could effectively scavenge the initiating free radicals of lipid peroxidation. On the other hand, it seems probable that, because of its high solubility in the lipid phase, reserpine interferes with the propagation phase of the peroxidative process.

Inhibition of phosphorylation by reserpine may be involved with the molecular mechanism of action of the drug. Phenothiazine derivatives, e.g. trifluperazine, have been shown to inhibit calmodulin-dependent synaptic protein phosphorylation [13]. Whether the effects of reserpine on protein phosphorylation in cortical slices are mediated by alterations in calmodulin- or cAMP-dependent protein phosphorylation is difficult to predict from the results of the present experiments. However, these observations are significant in view of the fact that both the protein phosphorylation and the lipid peroxidation can modify various membrane phenomena [14, 15].

In summary, results of our experiments show that reserpine is an effective inhibitor of both lipid peroxidation and protein phosphorylation in rat brain. Presumably, these effects are due to its action on the brain cell membrane.

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Structural assignment of an *N*-glucuronide metabolite of the phenylethanolamine *N*-methyltransferase (PNMT) inhibitor 1,2,3,4-tetrahydroisoquinoline-7-sulfonamide by ^{15}N-NMR*

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The compound 1,2,3,4-tetrahydroisoquinoline-7-sulfonamide (SK&F 29661) has been shown to inhibit phenylethanolamine *N*-methyltransferase (PNMT) in animals [1]. After oral administration of [^{14}C]SK&F 29661 to the dog (15 mg/kg), about 90% of the radiolabel in urine is excreted as SK&F 29661 with 5–10% of the urinary radiolabel identified as a conjugate of SK&F 29661 [2]. Data from enzymatic hydrolysis and mass spectrometry indicated that the conjugate is a glucuronide of SK&F 29661. This conjugate is hydrolyzed by β-glucuronidase to SK&F 29661. Hence, it is an *N*-glucuronide and not a *C*-glucuronide, since *C*-glucuronides are chemically stable and remain intact after incubation with β-glucuronidase [3, 4]. However, the position of glucuronidation, whether at the ring nitrogen as in **1** or at the sulfonamide nitrogen as in **2**, could not be established from these studies (Fig. 1). Since we had only a limited amount of the *N*-glucuronide, we sought a nondestructive method to differentiate these two isomers. ^{1}H-NMR was attempted to detect and integrate the sulfonamide and amine protons of the *N*-glucuronide, but was not successful due to the interference of water peak and uncertainty of the chemical shifts of the amine protons. This report describes a unique application of ^{15}N-NMR to assign unequivocally the position of glucuronidation in drug metabolism studies.

[^{15}N]SK&F 29661 was synthesized according to published procedures [1, 5]. 2-Acetyl-1,2,3,4-tetrahydroisoquinoline was reacted with chlorosulfonic acid to give 2-acetyl-1,2,3,4-tetrahydroisoquinoline-7-sulfonyl chloride which was treated with $^{15}NH_4OH$ (99%) to form 2-acetyl-1,2,3,4-tetrahydroisoquinoline-7-sulfonamide. Hydrolysis of the sulfonamide with 18% hydrochloric acid gave [^{15}N]SK&F 29661 hydrochloride with ^{15}N-enrichment at the sulfonamide nitrogen.

Materials and methods

$^{15}NH_4OH$ (99%). This was purchased from MSD Isotopes, Merck Chemical Division.

[^{14}C]*SK&F* 29661. This was prepared and provided by the Chemical Technology Department of Smith Kline & French Laboratories according to literature procedures [6].

* Preliminary results were presented at the 1984 International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 16–21, 1984.

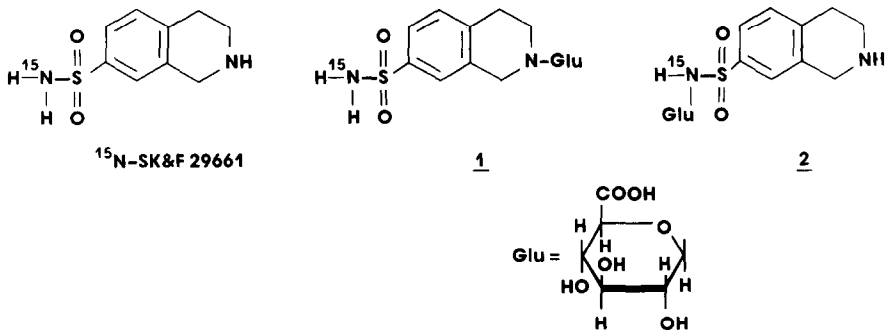

Fig. 1. Chemical structures of SK&F 29661 and the glucuronides.

The specific activity of [^{14}C]SK&F 29661 was 14.3 μCi/mg.

Isolation and purification of the metabolite. A 9 to 1 mixture of [^{15}N]SK&F 29661 and [^{14}C]SK&F 29661 was administered orally (25 mg/kg) to two female Beagle dogs, and 0–24 hr urine was collected. Each dog received about 75 μCi of ^{14}C-radioactivity. A sample of 88 ml of 0–24 hr dog urine was passed through a 70 g reverse phase C_{18} column (Alltech Associates, Adsorbosil C_{18} 100/200 mesh). Water was first used to remove the inorganic components, and methanol was then used to remove the organic components. The methanol extract was concentrated to give a residue which was chromatographed through a silica gel column (E. Merck, silica gel 60, 7–230 mesh). The column (2 cm × 12 cm) was first eluted with ethyl acetate–methanol–water–ammonia, 100:20:10:1. The proportion of methanol was then increased from 20 to 30, 40, 50 and finally 60. TCL (silica gel, methanol–ethyl acetate–ammonia, 60:40:5) was used to determine which fractions contained [^{15}N]SK&F 29661 and [^{15}N]SK&F 29661-glucuronide. Fractions were combined and further purified by HPLC using a Whatman Partisil 10 ODS-3 Magnum 9 column (25 cm × 9.4 mm), with u.v. set at 275 nm and a flow rate of 3 ml/min. The mobile phase used to purify [^{15}N]SK&F 29661 isolated from urine consisted of 100% 0.05 M ammonium acetate buffer, while a solvent system of 10% methanol in 0.05 M ammonium acetate was used for [^{15}N]SK&F 29661-glucuronide.

Enzymatic hydrolysis of [^{15}N]*SK&F 29661-glucuronide.* The purified metabolite was dissolved in a buffer solution, mixed with enzyme preparation, and incubated at 37° overnight along with a control where no enzyme preparation was added. Enzymes used were β-glucuronidase (type H-2) from *Helix pomatia* (0.08 M, pH = 5.2, phosphate buffer), β-glucuronidase from bovine liver (0.1 M, pH = 6.8, phosphate buffer) and sulfatase (type VI) from *Aerobacter aerogenese* (0.1 M, pH = 7.4, Tris/HCl buffer). The hydrolysis was followed by an HPLC system consisting of a Whatman Partisil 5, ODS-3, RAC column, with u.v. set at 280 nm and a flow rate of 2.0 ml/min. The buffer used was 0.05 M, pH 4, ammonium acetate, and the mobile phase used was MeOH–buffer, 5:100.

Fast atom bombardment mass spectra (FAB–MS). The spectra were obtained using a Finnigan-MAT triple stage quadruple (TSQ) mass spectrometer equipped with a 4500 series ion source and an Ion Tech 11NF saddle field gun. Xenon was used as the primary ionizing beam and glycerol as the target matrix.

^{15}N-NMR and ^{13}C-NMR spectra. ^{15}N-NMR spectra (8.059 MHz) were acquired on a Varian FT-80 spectrometer at 29.5° in DMSO-d_6 solution with $^{15}NH_4NO_3$ as an external standard. A pulse width of 15 μsec (60° flip angle), 8K data points, and 8000 Hz spectral width were used, and 500–40,000 transients per spectrum were accumulated depending on the concentration of the sample. About 30 mg of the recovered [^{15}N]SK&F 29661 and 20 mg of the *N*-glucuronide were used for the measurements. ^{13}C-NMR spectra were acquired using a Varian CFT-20 spectrometer at 30° in D_2O/Dioxane with a flip angle 45°, sweep width 4000 *Hz* and 8K data points.

Results and discussion

A mixture of [^{15}N]- and [^{14}C]SK&F 29661 was administered to Beagle dogs. Approximately 80% of the administered radiolabel was excreted in the 0–24 hr urine. TLC analysis of the urine demonstrated that about 90% of the urinary radiolabel was SK&F 29661, while about 10% was a single more polar metabolite. Both the SK&F 29661 and the metabolite isolated from the urine were purified by semipreparatory HPLC for enzymatic and structural analysis.

The purified metabolite was hydrolyzed to SK&F 29661 by β-glucuronidase from *H. pomatia* or from bovine liver but not by sulfatase from *A. aerogenese*. The hydrolyzed product showed an identical HPLC retention time to that of SK&F 29661. These results indicated that the metabolite was a *N*-glucuronide conjugate. ^{13}C-NMR data of the metabolite showed chemical shifts in the 70–100 ppm region which were attributed to the carbons from the sugar moiety. FAB–MS data displayed a peak at m/z 432 for negative mode corresponding to $(M-H)^-$ of the disodium salt of [^{15}N]SK&F 29661-glucuronide or m/z 434 for positive mode corresponding to $(M + Na)^+$ of the monosodium salt of [^{15}N]SK&F 29661-glucuronide.

Proton-coupled ^{15}N-NMR of [^{15}N]SK&F 29661·HCl (about 50 mg synthetic sample) gave a triplet at 69.0 ppm (J = 80.5 Hz) downfield from the reference $^{15}NH_4NO_3$ (Fig. 2). When ^{15}N-NMR spectra of [^{15}N]SK&F 29661 and its *N*-glucuronide, both isolated from dog urine as the free

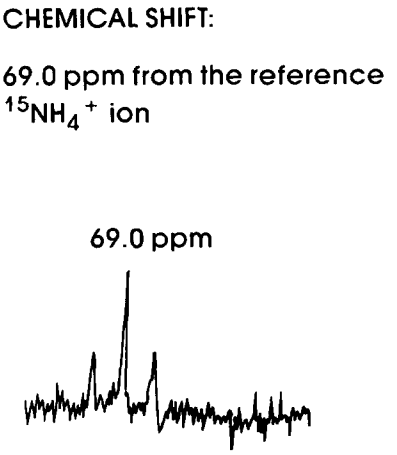

Fig. 2. Proton-coupled ^{15}N-NMR of [^{15}N]SK&F 29661·HCl (synthetic sample) in DMSO-d_6.

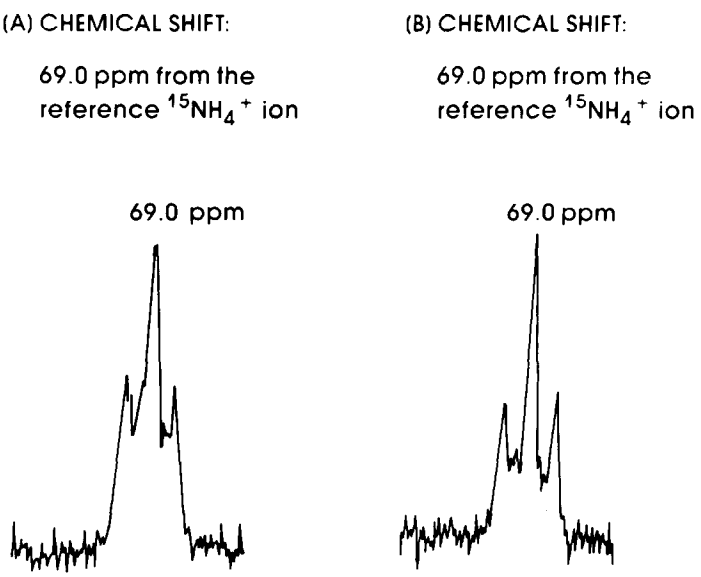

Fig. 3. Proton-coupled ^{15}N-NMR of (A) [^{15}N]SK&F 29661 and (B) [^{15}N]SK&F 29661-glucuronide in DMSO-d_6 with a drop of 2 N HCl added.

base form, were acquired under the same experimental conditions, a broad singlet was observed in both samples. This was presumably caused by the rapid exchange of sulfonamide protons with water in DMSO-d_6 on the NMR time scale [7, 8]. When the same samples in DMSO-d_6 were made acidic by adding a drop of 2 N HCl, both samples showed a triplet at 69.0 ppm (J = 80.5 Hz) downfield from the reference (Fig. 3). These results indicated that the position of glucuronidation of SK&F 29661 was at the ring nitrogen as in **1** and not at the sulfonamide nitrogen as in **2**, since proton-coupled ^{15}N-NMR of **2** would show a doublet rather than a triplet. Stable isotopes have been used frequently in the study of quantitative and qualitative investigations of metabolism by mass spectrometry [9]. However, few studies have been reported in which ^{15}N-NMR was used for structural determination [7, 10, 11]. Kanamori and Roberts [7] used ^{15}N-NMR to study the binding of cyanate and benzenesulfonamide to the active-site zinc of human carbonic anhydrase. Coxon *et al.* [10] used ^{15}N-NMR to study the structures of monoxanthen-9-yl derivatives of urea. Natural-abundance ^{15}N-NMR was used by Douglas [11] to characterize the structures of the intermediates in Fischer indolization reaction. Here we have shown another application of ^{15}N-NMR spectroscopy to differentiate between structural isomeric pairs encountered in drug metabolism studies. The NMR experiment was straightforward, and the results were unequivocal. Also, the sample could be recovered by injecting into a HPLC system. However, ^{15}N-enriched sample was required, and the amount of sample needed was in the range of 10–20 mg.

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