Characterization of Amino Acids and Steroids by Fluorine-19 Nuclear Magnetic Resonance Spectrometry of *p*-Fluorobenzoyl Derivatives

M. P. Spratt, Y. Meng,[1] and H. C. Dorn*

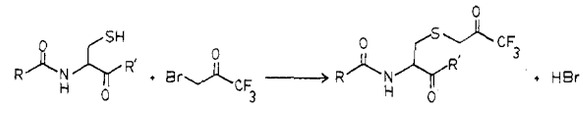
Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

In this paper, the utility of *p*-fluorobenzoyl chloride as an analytical ^{19}F NMR reagent to characterize sterols and amino acids is reported. The ^{19}F chemical shift data for approximately 30 sterols and amino acids are presented. The interaction of the *p*-fluorobenzoyl group with the steroid ring system makes a significant contribution to the ^{19}F chemical shift of these steroid derivatives. These results suggest a convenient technique for characterizing different steroids (e.g., estrogens, pregnanes, androstanes, etc.).

For several years, fluorinated derivatives have been used by a number of groups to analyze biological compounds. Cairns and co-workers (*1*) quantitatively characterized trifluoroacetate derivatives of hydroxyestrogens in complex mixtures derived from pregnant mare's urine by using gas chromatography/chemical ionization mass spectrometry (GC/MS). Ehrsson and Walle (*2–5*) have used fluorinated derivatives to quantitatively analyze pharmaceuticals by gas chromatography (GC) and mass spectrometry (MS). However, spectroscopic examination of complex biological mixtures by nuclear magnetic resonance (NMR) seldom permits speciation, due to excessive spectral overlap of closely related compounds. This is particularly true in ^{1}H NMR analysis where extremely complex spectra are observed for most macromolecules.

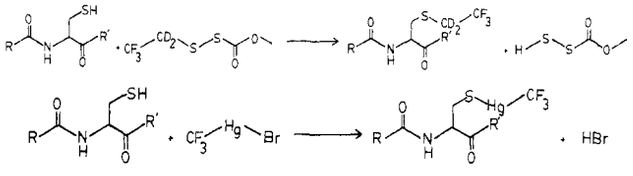
An alternate approach that easily complements ^{1}H and/or ^{13}C NMR data for complex biological systems is ^{19}F NMR. One advantage of ^{19}F NMR is that few biological systems of interest contain fluorine, thus, reducing a potentially significant background problem. Also, the ^{19}F nucleus is much more sensitive to subtle changes in chemical structure relative to the ^{1}H nucleus. This is indicated by the large ^{19}F NMR chemical shift range (~375 ppm) for a large number of compounds.

A number of papers have appeared in the literature concerning the use of ^{19}F NMR and fluorine derivatizing reagents for analyzing amino acids in proteins and peptides. An effective sulfhydryl specific fluorinating reagent for proteins and peptides is 3-bromo-1,1,1-trifluoropropanone (BrTFA). Brown and Seamon (*6*) have demonstrated that BrTFA is a simple and quantitative method for characterizing cysteine.



Other groups have used the BrTFA reagent to tag active site cysteines of glyceraldehyde 3-phosphate dehydrogenase (*7, 8*) which is a key enzyme in the glycolytic cycle. In addition, the cooperative interactions in hemoglobin (*9*) were studied by tagging the cysteine-93 residue with BrTFA. The ^{19}F chemical shifts for the BrTFA group have also been shown to be dependent on the conformation of surrounding amino acids in the protein complex (*10*) and the pH of the aqueous media containing the protein or enzyme (*11*).

Bendall and Lowe (*12, 13*) have studied active sites of papain by preparing derivatives of these enzymes utilizing *SS*-(2,2,2-trifluoro-1,1-dideuterioethyl) *O*-methyl dithioperoxycarbonate and trifluoromethylmercuric bromide as illustrated below:



Huestis and co-workers (*14*) have utilized ethyl thioltrifluoroacetate to specifically derivatize lysine residues 1 and 7 on ribonuclease S. In a similar study, Paselk and Levy (*15*) also used ethyl thioltrifluoroacetate to study glycine, phenylalanine, and the ξ-amino group of lysine in insulin.

Gaffield and Lundin (*16*) have used hexafluoroacetone derivatives and ^{19}F NMR as a means for determining nitrosoaminoalcohols and amines in foods. Zuber and co-workers (*17*) have also used pentafluoropropionic anhydride to analyze pharmaceuticals by ^{19}F NMR. However, only a paucity of published reports are in the literature regarding the use of fluorinated derivatives to characterize steroids by ^{19}F NMR. One example, is the study of Bayer and co-workers (*18*) where the trifluoroacetate derivatives of hydroxypregnane were characterized by ^{19}F NMR. They found a ^{19}F chemical shift range of approximately 0.9 ppm.

EXPERIMENTAL SECTION

Apparatus. JEOL PS-100 and JEOL FX-200 nuclear magnetic resonance spectrometers were used to obtain ^{19}F nuclear magnetic resonance (NMR) spectra at 94.08 and 187.7 MHz, respectively, for the model compounds. Both spectrometers operate in the Fourier transform (FT) mode. The NMR spectrometers were used with internal deuterium lock systems operating at 15.14 and 30.3 MHz, respectively. 1,2-Difluorotetrachloroethane (Peninsular Chem Research) was uses as the ^{19}F chemical shift reference with chloroform-*d* as solvent. Chemical shifts (δ ^{19}F) were measured in parts per million (ppm) with a negative value indicating shielding relative to the reference.

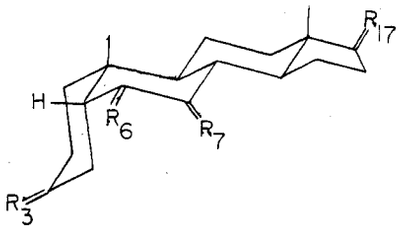
Procedures. *Preparation of Steroid p-Fluorobenzoates.* In a flask 0.1 mmol of the steroid was added to 10 mL of anhydrous pyridine (distilled and stored over KOH pellets). A 10% molar excess of the equivalent amount of *p*-fluorobenzoyl chloride was slowly added. The reaction mixture was then stirred for 24 h at room temperature under nitrogen. After 24 h the pyridine was removed by vacuum distillation. The remaining residue was redissolved in 25 mL of ethyl acetate and washed twice with 20 mL of 5% $NaHCO_3$ and once with 20 mL of distilled H_2O. The ethyl acetate was dried over $MgSO_4$ and removed in vacuo.

An optional technique used to isolate the product employed dilution of the reaction mixture with 20 mL of a 50/50 benzene/ethyl acetate solution. This was washed twice with 20 mL

[1] Permanent address: Nanjing College of Pharmacy, Nanjing, Jianqsu, The People's Republic of China.

0003-2700/85/0357-0076$01.50/0

Table I. ^{19}F NMR Chemical Shifts for *p*-Fluorobenzoate Derivatives of 5β-Steroids



STEROID	δ_F, PPM R_3	R_6	R_7	R_{20}
5β-androstan-3β-ol-17-one	-38.89(β)			
5β-androstan-3α-ol-17-one	-38.78(α)			
5β-pregnan-3α,6α-diol-20-one	-38.41(α)[1]	-38.51(α)[1]		
5β-pregnan-3α,20α-diol	-38.77(α)[1]			-38.82(α)[1]
5β-cholanic acid-3α,7α-diol	-38.78(α)[1]		-37.58(α)[1]	

1) ^{19}F Chemical Shifts have been tentatively assigned.

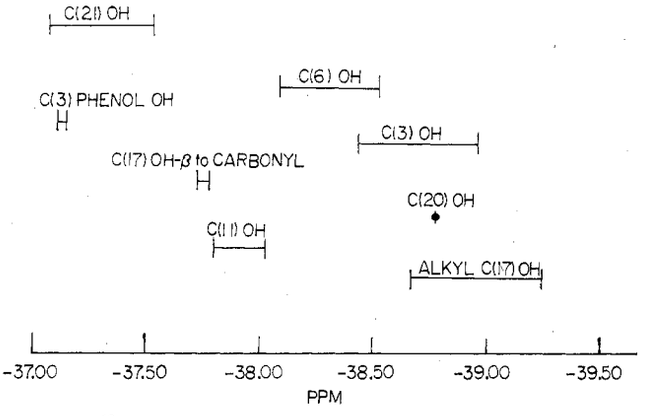


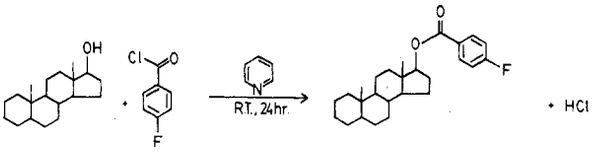
Figure 1. ^{19}F chemical shift range for *p*-fluorobenzoyl derivatives of sterols.

of 5% $NaHCO_3$ and three times with 1 M $CuSO_4$ which complexes with the pyridine and forms a dark blue precipitate. The remaining solvent was washed once with distilled water, dried over $MgSO_4$, and removed in vacuo. The resulting material in most cases was a crude solid which was purified by recrystallization from ethyl acetate. Yields were highly dependent on whether the hydroxy group of the steroid was primary, secondary, or tertiary (*19*).

Preparation of N-p-Fluorobenzoyl Amino Acid Derivatives. The method used to derivatize amino acids with *p*-fluorobenzoyl chloride was similar to the derivatization technique developed by Kingston and LeFevre (*20*). The general procedure is as follows: The amino acid model (1 mmol) was added to 10 mL of 1 N NaOH and stirred with a magnetic stirrer until dissolved. To this solution 10 mL of ethyl ether was added followed by slow addition of 5 mL of ethyl ether containing 1.1 mmol (or a 10% excess of the equivalent amount in cases of more than one functional group) of *p*-fluorobenzoyl chloride. The aqueous and ethyl ether solutions were mixed and the pH of the reaction mixture was occasionally checked to assure that the pH was always greater than 10. If the pH was less than 10, 1 N NaOH was added in dropwise fashion to obtain a pH ≥10. After the *p*-fluorobenzoyl chloride was added, the heterogeneous solution was vigorously stirred at room temperature for a 24-h period. After 24 h the solution was placed in a separatory funnel and acidified with 6 N HCl to a pH of 1. The solution was extracted three times with 20-mL portions of ethyl acetate. The organic portions were combined, dried over $MgSO_4$, and then removed in vacuo. The resulting residue in most cases was a crystalline solid. Upon recrystallization (ethyl acetate/hexane), all samples yielded white crystalline solids.

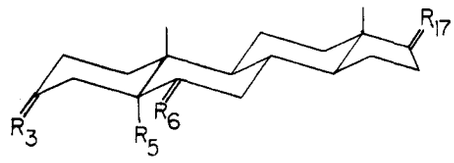
RESULTS AND DISCUSSION

The general reaction for the preparation of the *p*-fluorobenzoate derivatives is illustrated below:



The ^{19}F chemical shifts for a number of *p*-fluorobenzoate sterol derivatives are presented in Table I–V and illustrated in Figure 1. The ^{19}F spectral assignments for some of the di- and tri-*p*-fluorobenzoate steroid derivatives in Tables I–V are tentative (e.g., cholestane-3β,5α,6β-triol). However, in some cases ^{19}F spectral assignments were based on the relative intensities of the ^{19}F signals obtained when a *limiting* amount of the *p*-fluorobenzoyl chloride was used in the derivative preparation. As an example, one would expect (and observes) a more rapid reaction at the C21 position (primary alcohol) as opposed to the α-C17 position (tertiary alcohol) in Δ^4-

Table II. ^{19}F NMR Chemical Shifts for *p*-Fluorobenzoate Derivatives of 5α-Steroids



STEROID	R_3	R_5	R_6	R_{17}	R_{20}
	δ_F, PPM				
5α-androstan-3β-ol-17-one	-38.90(β)				
5α-androstan-3α-ol-17-one	-38.73(α)				
5α-androstan-17β-ol-3-one				-38.66(β)	
5α-pregnan-3β,20β-diol	-38.86(β)[1]				-38.83(β)[1]
cholestane-3β,5α,6β-triol	-38.55(β)[1]	-38.69(α)[1]	-38.10(β)[1]		

1) ^{19}F Chemical Shifts have been tentatively assigned.

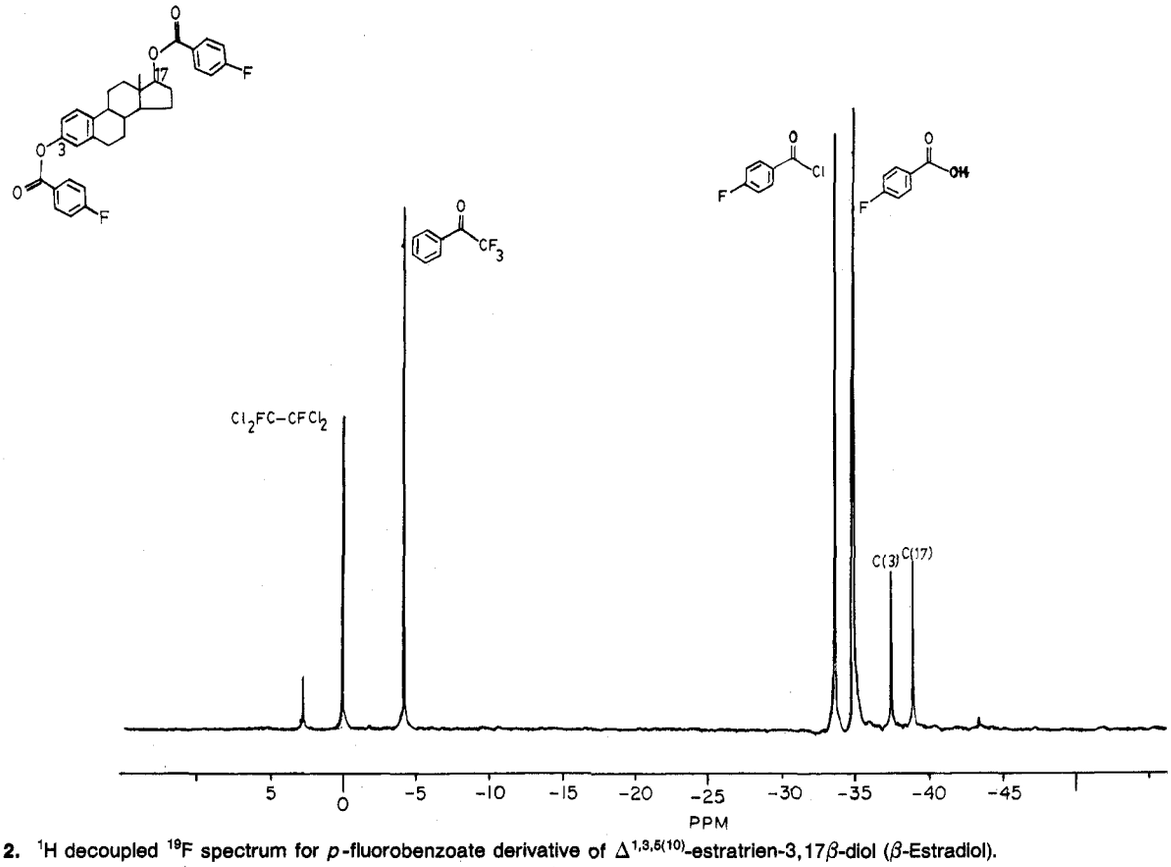
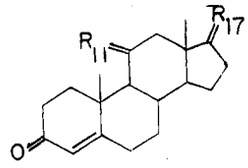


Figure 2. ^1H decoupled ^{19}F spectrum for *p*-fluorobenzoate derivative of $\Delta^{1,3,5(10)}$-estratrien-3,17β-diol (β-Estradiol).

pregnen-17α,21-diol-3,20-dione (Table III). Where appropriate, the ^{19}F spectral assignments are indicated as they appear in Tables I–V. The range of ^{19}F chemical shifts for the *p*-fluorobenzoates of the sterols is approximately twice the range found for the trifluoroacetates for a closely related sterol model set (*18*).

Table III. ^{19}F NMR Chemical Shifts for *p*-Fluorobenzoate Derivatives of Δ^4-Steroids



STEROID	R_{11}	δ_F, PPM R_{17}	R_{21}
Δ^4-pregnen-21-ol-3,20-dione	R_{11} = ; R_{17} =		-37.54
Δ^4-pregnen-21-ol-3,11,20-trione	R_{11} = =O ; R_{17} =		-37.49
Δ^4-pregnen-11β,21-diol-3,20-dione	R_{11} = ; R_{17} =	-38.02(β)	-37.37
Δ^4-pregnen-17α,21-diol-3,20-dione	R_{11} = ; R_{17} =	-37.73(α)[1]	-37.34[1]
Δ^4-pregnen-17α,21-diol-3,11,20-trione	R_{11} = =O ; R_{17} =	-37.77(α)[1]	-37.15[1]
Δ^4-pregnen-11β,17α,21-triol-3,20-dione	R_{11} = ; R_{17} =	-37.81(β)[1] -37.74(α)[1]	-37.10[1]
Δ^4-androsten-17β-ol-3-one	R_{11} = ; R_{17} =	-38.63(β)	

1) ^{19}F Chemical Shifts have been tentatively assigned.

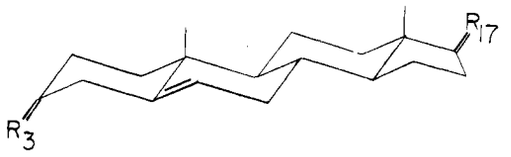
In Tables I and II are summarized the ^{19}F chemical shifts for a number of C3 substituted steroids with the A/B ring system having cis and trans geometries at the C5 ring junctions, respectively. The ^{19}F chemical shifts are nearly insensitive to the cis and trans geometries at the C5 ring junction (e.g., 5β-androstan-3β-ol-17-one vs. 5α-androstan-3β-ol-17-one). However, examination of the four isomers of androstan-3-ol-17-one reveals that the ^{19}F chemical shifts for these *p*-fluorobenzoates allow spectral identification of the α or β isomer at the C3 carbon. For example, the ^{19}F chemical shift for 5β-androstan-3β-ol-17-one is −38.89 ppm while for 5β-androstan-3α-ol-17-one the ^{19}F resonance is −38.78 ppm. One observes the same ^{19}F chemical shift trend for the 5α-androstane isomers (e.g., 5α-androstan-3β-ol-17-one, −38.90 ppm and 5α-androstan-3α-ol-17-one, −38.73 ppm).

The introduction of a second *p*-fluorobenzoyl group at the C6 position typically has a deshielding influence on the C3 *p*-fluorobenzoate ^{19}F chemical shift (e.g., 5β-pregnan-3α,6α-diol-20-one). The observation of a deshielding effect by introduction of a second aromatic ring is similar to data obtained in simple model systems (*19*). The influence is apparently not observed when the second *p*-fluorobenzoyl group is introduced at the C7 and/or a position further removed from C3.

The ^{19}F chemical shifts for a number of Δ^4-steroids are presented in Table III. One major feature of the data in Table III is the influence of a β-carbonyl group on the ^{19}F chemical shift of the *p*-fluorobenzoate group which causes a significant deshielding of the ^{19}F nucleus. For example, comparing the ^{19}F chemical shift of the C21 *p*-fluorobenzoate ester of Δ^4-pregnen-21-ol-3,20-dione (−37.54 ppm) to the *n*-butyl *p*-fluorobenzoate ester (−38.86 ppm) (*19*), one observes a deshielding of the ^{19}F nucleus by 1.32 ppm. The significant deshielding of the ^{19}F nucleus by the β-carbonyl functional group was expected in light of our previous study (*19*). In this study it was found that *p*-fluorobenzoyl anhydride derivatives of carboxylic acids had ^{19}F chemical shifts significantly more deshielded than any other alcohol derivative (~3.5 ppm). This effect was also observed for trifluoroacetates of C21 sterols containing a β-carbonyl group (*18*).

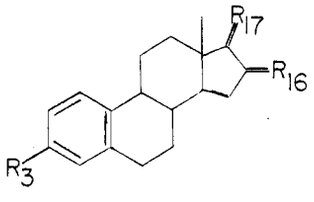
For the sterols with a tertiary alcohol group at C17 (e.g., Δ^4-pregnen-17α-21-diol-3,20-dione), the ^{19}F chemical shifts for these C17 *p*-fluorobenzoate groups are much lower (more deshielded) than normally encountered for tertiary alcohol derivatives. For example, the ^{19}F chemical shift for the *p*-fluorobenzoate of *tert*-butyl alcohol is −39.69 ppm (*19*). However, the presence of the β-carbonyl group and the second *p*-fluorobenzoate ring in reasonably close proximity substantially deshield the *p*-fluorobenzoate group at C17. Thus, the relatively constant ^{19}F shifts of approximately −37.7 ppm at C17 are understandable in terms of these factors. It is also interesting to note that the ^{19}F chemical shift for the Δ^4-androstan-17β-ol-3-one *p*-fluorobenzoate derivative is in fair agreement with the value obtained for the simple model, cyclohexanol (−38.88 ppm) (*19*). The *p*-fluorobenzoate derivatives at the secondary C11 position are substantially de-

Table IV. ^{19}F NMR Chemical Shifts for *p*-Fluorobenzoate Derivatives of Δ^5-Steroids



STEROID	δ_F, PPM R_3	R_{17}
Δ^5-cholesten-3ß-ol (cholesterol)	-38.90(ß)	
Δ^5-androsten-3ß-ol-17-one	-38.86(ß)	
Δ^5-pregnen-3ß-ol-20-one (pregnenolone)	-38.71(ß)	
17α-methyl-5-androsten-3ß,17ß-diol (methylandrostenediol)	-38.74(ß)	-39.24(ß)

Table V. ^{19}F NMR Chemical Shifts for *p*-Fluorobenzoate Derivatives of $\Delta^{1,3,5}$-Estratriene Steroids

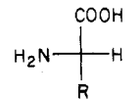


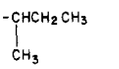
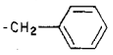
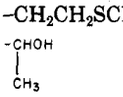
STEROID	δ_F, PPM R_3	R_{16}	R_{17}
$\Delta^{1,3,5}$-estratrien-3-ol-17-one (Estrone)	-37.19		
$\Delta^{1,3,5}$-estratrien-3,17ß-diol (ß-Estradiol)	-37.19		-38.67(ß)
$\Delta^{1,3,5}$-estratrien-3,16α,17ß-triol (Estriol)	-37.14	-37.94(α)[1]	-38.08(ß)[1]

1) ^{19}F Chemical Shifts have been tentatively assigned.

shielded for Δ^4-pregnen-11β-21-diol-3,20-done and Δ^4-pregnen-11β,17α,21-triol-3,20-dione with ^{19}F chemical shifts of −38.02 and −37.81 ppm, respectively. This could be due in part to the aromatic ring interactions of the second and third aromatic systems with the *p*-fluorobenzoate ring at the C11 position.

Table VI. ^{19}F NMR Chemical Shifts for *N*-*p*-Fluorobenzoate Derivatives of Amino and Nucleic Acids



R	amino acid	δ_F, ppm
$-CH_3$	D,L-alanine	−41.05
$-CH(CH_3)_2$	D,L-valine	−41.14
$-CH_2CH(CH_3)_2$	D,L-leucine	−41.07
	D,L-isoleucine	−41.10
	D,L-phenylalanine	−40.91
$-CH_2CH_2SCH_3$	D,L-methionine	−40.85
	threonine	−40.78
		−37.15 2° (OH)

In Table IV several Δ^5-steroid *p*-fluorobenzoate derivatives are reported. Once again, the narrow range (−39.90 to −39.71 ppm) of ^{19}F chemical shifts for the C3 *p*-fluorobenzoate group is very similar to the simple cyclohexanol derivative (−38.88 ppm) (*19*). In addition, the C17 ^{19}F chemical shift for the 17α-methyl-5-androstan-3β,17β-diol derivative is close to the expected value for tertiary alcohol derivatives.

The sterols in Table V are easily characterized by the phenolic C3 *p*-fluorobenzoate ^{19}F resonance at approximately −37.19 ppm for estrone, β-estradiol, and estriol. As Figure 2 clearly shows, the two ^{19}F resonances for the phenol (−37.19 ppm) and the C17 −37.2 alcohol (−38.67 ppm) groups for β-estradiol are well resolved in the ^1H decoupled ^{19}F spectrum and can be easily assigned. The estriol is easily characterized by the C16 and C17 secondary *p*-fluorobenzoate ^{19}F resonances at −37.98 and −38.08 ppm, respectively. In similar manner to other systems discussed (vide supra) vicinal *p*-fluorobenzoate ring interactions at C16 and C17 deshield the ^{19}F nucleus.

A limited number of amino acids were also characterized by using *p*-fluorobenzoyl chloride. The ^{19}F chemical shift data for the *p*-fluorobenzamides of the amino acids are listed in Table VI. The ^{19}F chemical shifts observed are characteristic for primary amine derivatives (*19*). The overall ^{19}F chemical shift range for the *p*-fluorobenzamides is only ~0.36 ppm for the limited number of amino acids examined. These results reflect the fact that the ^{19}F test nucleus is eight to nine bonds removed from the structural changes on the amino acid.

In spite of the large interatomic distances between the fluorine test nucleus and the substrate changes of the amino acid, it is still possible to observe the effect of alkyl or phenyl substitution on alanine. These effects (although greatly attenuated) are similar to the results previously obtained for alcohol derivative (*19*). The ^{19}F chemical shift data can also be used to characterize amino acids containing other functional group such as threonine where the hydroxyl functional groups are also derivatized (Table VI).

CONCLUSION

The results of the present study demonstrate the potential utility of ^{19}F NMR for characterizing sterols and amino acids. That is, the ^{19}F chemical shift parameter for *p*-fluorobenzoyl derivatives is sufficiently sensitive to allow spectral identification of several types of sterols and even differences in structure at the β-position of amino acids. The ^{19}F chemical shifts are influenced by (1) the position of *p*-fluorobenzoate on the steroid ring system, (2) the type of hydroxyl group (e.g., primary, secondary, or tertiary alcohols), (3) ring interactions between mono-, di-, and tri-*p*-fluorobenzoate groups, and (4) substituent effects for groups in close proximity to the *p*-fluorobenzoate group (e.g., β-carbonyl). Although ^{19}F spectral overlap of a few steroid derivatives is observed, nevertheless, unique ^{19}F shifts are obtained for most of the sterols. Thus, the modest range of ^{19}F chemical shifts (~2 ppm for all hydroxyl groups) and ease of derivative preparation suggest an important role for *p*-fluorobenzoyl chloride in characterizing sterols.

Registry No. *p*-Fluorobenzoyl chloride, 403-43-0; 5α-androstan-3β-ol-17-one *p*-fluorobenzoate, 92010-48-5; 5α-androstan-3α-ol-17-one *p*-fluorobenzoate, 92010-49-6; 5α-androstan-17β-ol-3-one *p*-fluorobenzoate, 92010-50-9; 5α-pregnan-3β,20β-diol di-*p*-fluorobenzoate, 92010-51-0; cholestane-3β,5α,6β-triol tri-*p*-fluorobenzoate, 92010-52-1; 5β-androstan-3β-ol-17-one *p*-fluorobenzoate, 92010-53-2; 5β-androstan-3α-ol-17-one *p*-fluorobenzoate, 92010-54-3; 5β-pregnan-3α,6α-diol-20-one di-*p*-fluorobenzoate, 92010-55-4; 5β-pregnan-3α,20α-diol di-*p*-fluorobenzoate, 92010-56-5; 5β-cholanic acid-3α,7α-diol di-*p*-fluorobenzoate, 92054-20-1; Δ^4-pregnen-21-ol-3,20-dione *p*-fluorobenzoate, 92010-57-6; Δ^4-pregnen-21-ol-3,11,20-trione *p*-fluorobenzoate, 92010-58-7; Δ^4-pregnen-11β,21-diol-3,20-dione di-*p*-fluorobenzoate, 92010-59-8; Δ^4-pregnen-17α,21-diol-3,20-dione di-*p*-fluorobenzoate, 92010-60-1; Δ^4-pregnen-17α,21-diol-3,11,20-trione di-*p*-fluorobenzoate, 92010-61-2; Δ^4-pregnen-11β,17α,21-triol-3,20-dione tri-*p*-fluorobenzoate, 92010-62-3; Δ^4-androsten-17β-ol-3-one *p*-fluorobenzoate, 92010-63-4; cholesterol *p*-fluorobenzoate, 59857-03-3; Δ^5-androsten-3β-ol-17-one *p*-fluorobenzoate, 92010-64-5; pregnenolone *p*-fluorobenzoate, 92010-65-6; methylandrostenediol *p*-fluorobenzoate, 92010-66-7; estrone *p*-fluorobenzoate, 92010-67-8; β-estradiol di-*p*-fluorobenzoate, 92010-68-9; estriol tri-*p*-fluorobenzoate, 92010-69-0; DL-alanine *N*-*p*-fluorobenzoate, 451-28-5; DL-valine *N*-*p*-fluorobenzoate, 92054-21-2; DL-leucine *N*-*p*-fluorobenzoate, 92010-70-3; DL-isoleucine *N*-*p*-fluorobenzoate, 92010-71-4; DL-phenylalanine *N*-*p*-fluorobenzoate, 92010-72-5; DL-methionine *N*-*p*-fluorobenzoate, 65054-76-4; threonine di-*p*-fluorobenzoate, 92010-73-6.

LITERATURE CITED

(1) Cairns, T.; Siegmund, E. G.; Rader, B. *Anal. Chem.* **1981**, *53*, 1217–1222.
(2) Ehrsson, H.; Walle, T.; Brotell, H. *Acta Pharm. Suec.* **1971**, *8*, 319–328.
(3) Walle, T.; Ehrsson, H. *Acta Pharm. Suec.* **1971**, *8*, 27–38.
(4) Walle, T.; Ehrsson, H. *Acta Pharm. Suec.* **1970**, *7*, 389–406.
(5) Ervik, M.; Walle, T.; Ehrsson, H. *Acta Pharm. Suec.* **1970**, *7*, 625–634.
(6) Brown, W. E.; Seamon, K. B. *Anal. Biochem.* **1978**, *87*, 211–222.
(7) Bode, J.; Blumenstein, M.; Raftery, M. A. *Biochemistry* **1975**, *14*, 1146–1152.
(8) Bode, J.; Blumenstein, M.; Raftery, M. A. *Biochemistry* **1975**, *14*, 1153–1160.
(9) Huestis, W. H.; Raftery, M. A. *Biochemistry* **1972**, *11*, 1648–1654.
(10) Seamon, K. B.; Hartshorne, D. J.; Bothner-By, A. A. *Biochemistry* **1977**, *16*, 4039–4046.
(11) Bendall, M. R.; Lowe, G. *Eur. J. Biochem.* **1976**, *65*, 481–491.
(12) Bendall, M. R.; Lowe, G. *Eur. J. Biochem.* **1976**, *65*, 493–502.
(13) Bendall, M. R.; Lowe, G. *FEBS Lett.* **1976**, *72*, 231–234.
(14) Huestis, W. H.; Raftery, M. A. *Biochemistry* **1971**, *10*, 1181–1186.
(15) Paselk, R. A.,; Levy, D. *Biochemistry* **1974**, *13*, 3340–3346.
(16) Gaffield, W.; Lundin, R. E. *IARC Sci. Publ.* **1978**, *19*, 87–95.
(17) Zuber, G. E.; Stalger, D. B.; Warren, R. J. *Anal. Chem.* **1983**, *55*, 64–67.
(18) Voelter, W.; Brietmaier, E.; Jung, G.; Bayer, E. *Org. Mag. Reson.* **1970**, *2*, 251–257.
(19) Spratt, M. P.; Dorn, H. *Anal. Chem.* **1984**, *56*, 2038–2043.
(20) LeFevre, J. W.; Kingston, D. G. I. *J. Org. Chem.*, in press.

RECEIVED for review April 13, 1984. Accepted July 2, 1984.