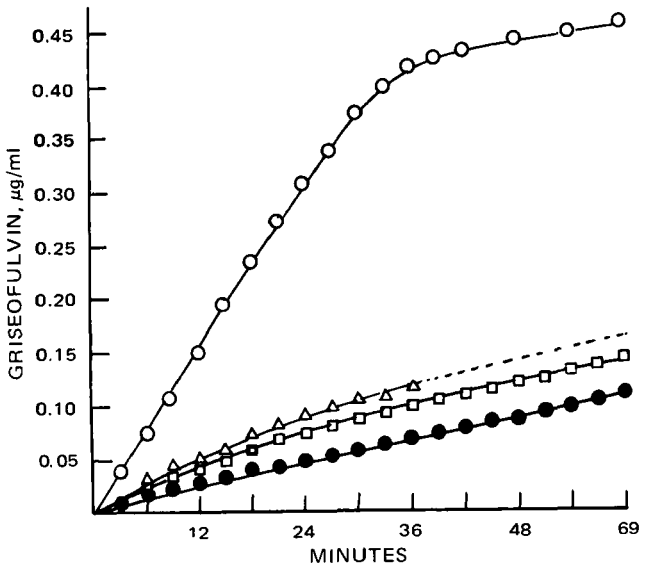

Figure 4—*Release of griseofulvin from constant surface tablets. Key: ○, 10% solid dispersion; △, 10% griseofulvin–90% succinic anhydride physical mixture; □, 10% griseofulvin–90% succinic acid physical mixture; and ●, pure griseofulvin.*

a marked increase in dissolution rate cannot be explained by the presence of a higher energy polymorphic form because the X-ray diffraction study showed that the identical crystalline form was present in the dispersed system as the pure griseofulvin used in the present study (5).

Since some succinic anhydride (about 7% w/w) was formed during the preparation of solid dispersions (5), it was thought that such an impurity might enhance the dissolution rate. The fact that the dissolution rate of griseofulvin was only moderately increased in the presence of 90% succinic anhydride (Fig. 4), however, rules out the important role of this decomposition product. Based on these results and analyses, it is postulated that the major factor in the increasing dissolution rate in the 10% griseofulvin dispersion was the presence of extremely fine crystals of griseofulvin. Crystalline size alone has been shown to affect the solubility of drugs (9).

After about 42 min, the cumulative plot curve of the 10% griseofulvin solid dispersion became parallel with that of pure griseofulvin. This result probably was due to the presence of negligible concentrations of the solubilizers, succinic acid and succinic anhydride, at the outer controlling layer of the tablet and to the presence of a coarser particle size of griseofulvin at the dissolution surface; this coarser particle size was formed as a result of the aggregation and/or crystal growth of ultrafine crystals. It must be noted that these tablet dissolution studies were carried out under essentially sink conditions (the solubility of griseofulvin at 37° is about 12.5 µg/ml).

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Application of Trichloroacetyl Isocyanate to NMR Analysis of Steroids of Pharmaceutical Interest I: Corticosteroids and Chemically Related Compounds

MONIQUE LANOUETTE, DONALD LEGAULT, and BRUCE A. LODGE [x]

Abstract □ The trichloroacetyl carbamates of 38 corticosteroids and chemically related compounds were prepared, and their NMR spectra in deuterochloroform were obtained. The effects of the introduction of a number of functional groups on the chemical shift of the carbamate proton signals were determined.

Keyphrases □ Trichloroacetyl isocyanate—reaction with corticosteroids to form carbamates, application to NMR analysis □ NMR—analysis, carbamates of corticosteroids formed by reaction with trichloroacetyl isocyanate □ Corticosteroids—NMR analysis of carbamates formed by reaction with trichloroacetyl isocyanate □ Carbamates—formed by reaction of trichloroacetyl isocyanate with corticosteroids, NMR analysis

Over the past several years, trichloroacetyl isocyanate has been used as a synthetic (1–6) and as a diagnostic (7–10) reagent. It reacts with hydroxyl groups to form carbamates of type I. Such derivatives are usually much more soluble in deuterochloroform than are the parent compounds; indeed, many underivatized corticosteroids

Table I—Chemical Shifts[a] (Hertz) of the Proton Signals in the NMR Spectra[b] of the Trichloroacetyl Carbamate Derivatives of Corticosteroids and Chemically Related Compounds

		Carbamate Protons						Carbinol Protons				Methyl Protons			Vinylic Protons			
	Compound	11β	17α	21	17β	11α	3β	11α	21	11β	3α	18	19	(Other)	1	2	4	6
I	11α-Hydroxypregn-4-ene-3,20-dione	—	—	—	—	523	—	—	—	323	—	46	80	128 (21)	—	—	346	—
II	11β-Hydroxypregn-4-ene-3,20-dione	508	—	—	—	—	—	336	—	—	—	52	79	128 (21)	—	—	343	—
III	11β-Hydroxyandrost-4-ene-3,17-dione	512	—	—	—	—	—	338	—	—	—	68	80	—	—	—	344	—
IV	11β-Hydroxyandrosta-1,4-diene-3,17-dione	520	—	—	—	—	—	342	—	—	—	67	78	—	421	377	364	—
V	17α-Hydroxypregn-4-ene-3,20-dione	—	562	—	—	—	—	—	—	—	—	44	72	131 (21)	—	—	345	—
VI	17α-Hydroxypregn-4-ene-3,11,20-trione	—	544	—	—	—	—	—	—	—	—	40	85	129 (21)	—	—	344	—
VII	21-Hydroxypregn-4-ene-3,20-dione	—	—	526	—	—	—	—	286	—	—	44	71	—	—	—	344	—
VIII	21-Hydroxypregn-4-ene-3,11,20-trione	—	—	528	—	—	—	—	285	—	—	43	85	—	—	—	344	—
IX	Pregnenolone	—	—	—	—	—	503	—	—	—	282	40	64	128 (21)	—	—	—	326
X	11β,21-Dihydroxypregn-4-ene-3,20-dione	510	—	529	—	—	—	336	287	—	—	56	79	—	—	—	344	—
XI	11β,21-Dihydroxypregn-4-ene-3,20-dione 21-acetate	503	—	—	—	—	—	335	285	—	—	54	79	—	—	—	343	—
XII	17α,21-Dihydroxypregn-4-ene-3,20-dione	—	594	547	—	—	—	—	302	—	—	50	72	—	—	—	344	—
XIII	17α,21-Dihydroxypregn-4-ene-3,20-dione 21-acetate	—	574	—	—	—	—	—	293	—	—	48	72	—	—	—	344	—
XIV	Cortisone[c]	—	583	549	—	—	—	—	302	—	—	47	85	—	—	—	345	—
XV	Cortisone acetate[d]	—	563	—	—	—	—	—	291	—	—	46	86	—	—	—	344	—
XVI	Prednisone[e]	—	590	550	—	—	—	—	301	—	—	49	86	—	465	367	364	—
XVII	Prednisone 21-acetate	—	576	—	—	—	—	—	291	—	—	48	86	—	465	368	364	—
XVIII	Hydrocortisone[d]	512	563	547	—	—	—	342	305	—	—	62	78	—	—	—	342	—
XIX	Prednisolone	520	566	548	—	—	—	344	304	—	—	64	77	—	424	374	361	—
XX	Prednisolone 21-acetate	513	547	—	—	—	—	344	292	—	—	62	78	—	424	377	363	—
XXI	Betamethasone[d]	522	606	545	—	—	—	333	290	—	—	59	87	62 (16β)	418	380	367	—
XXII	Betamethasone 21-acetate[d]	517	586	—	—	—	—	334	280	—	—	58	85	60 (16β)	416	378	366	—
XXIII	Betamethasone 21-valerate[d]	514	586	—	—	—	—	333	281	—	—	58	86	60 (16β)	416	378	366	—
XXIV	Betamethasone 17-valerate	520	—	528	—	—	—	340	280	—	—	59	88	60 (16β)	417	382	371	—
XXV	Beclomethasone 17-propionate	520	—	530	530	—	—	346	280	—	—	60	93	61 (16β)	418	382	369	—
XXVI	Beclomethasone 21-propionate[d]	520	581	—	—	—	—	344	282	—	—	59	92	71 (16β)	416	380	367	—
XXVII	Beclomethasone dipropionate	514	—	—	—	—	—	344	272	—	—	57	92	61 (16β)	416	381	368	—
XXVIII	Dexamethasone[f]	531	611	547	—	—	—	331	306	—	—	69	80	57 (16α)	412	375	365	—
XXIX	Dexamethasone 21-acetate[f]	529	576	—	—	—	—	331	295	—	—	69	82	57 (16α)	413	377	366	—
XXX	Methylprednisolone 21-acetate	514	548	—	—	—	—	345	291	—	—	61	77	68 (6α)	423	375	363	—
XXXI	Fluprednisolone	516	553	540	—	—	—	345	304	—	—	64	76	—	422	378	381	—
XXXII	Fludrocortisone acetate[d]	509	594	—	—	—	—	338	292	—	—	59	86	—	—	—	346	—
XXXIII	Fluperolone acetate[c]	520	582	—	—	—	—	344	281	—	—	60	92	—	417	380	367	—
XXXIV	Paramethasone 21-acetate[f]	519	553	—	—	—	—	346	296	—	—	69	75	56 (16α)	422	377	381	—
XXXV	Triamcinolone acetonide	524	—	527	—	—	—	337	302	—	—	55	74	—	416	382	370	—
XXXVI	Triamcinolone 16α,21-diacetate[f]	533	587	—	—	—	—	334	299	—	—	67	84	—	415	381	368	—
XXXVII	Triamcinolone hexacetonide	517	—	—	—	—	—	334	291	—	—	54	74	—	415	382	370	—
XXXVIII	Fluocinonide	519	—	—	—	—	—	336	292	—	—	52	73	—	413	383	388	—

[a] Chemical shifts downfield from tetramethylsilane (0 Hz). [b] All spectra obtained at a concentration of 40 mg/ml. [c] Sample required 1 hr to react completely. [d] Sample required 30 min to react completely. [e] Sample required 24 hr to dissolve. [f] Spectrum obtained after 24 hr.

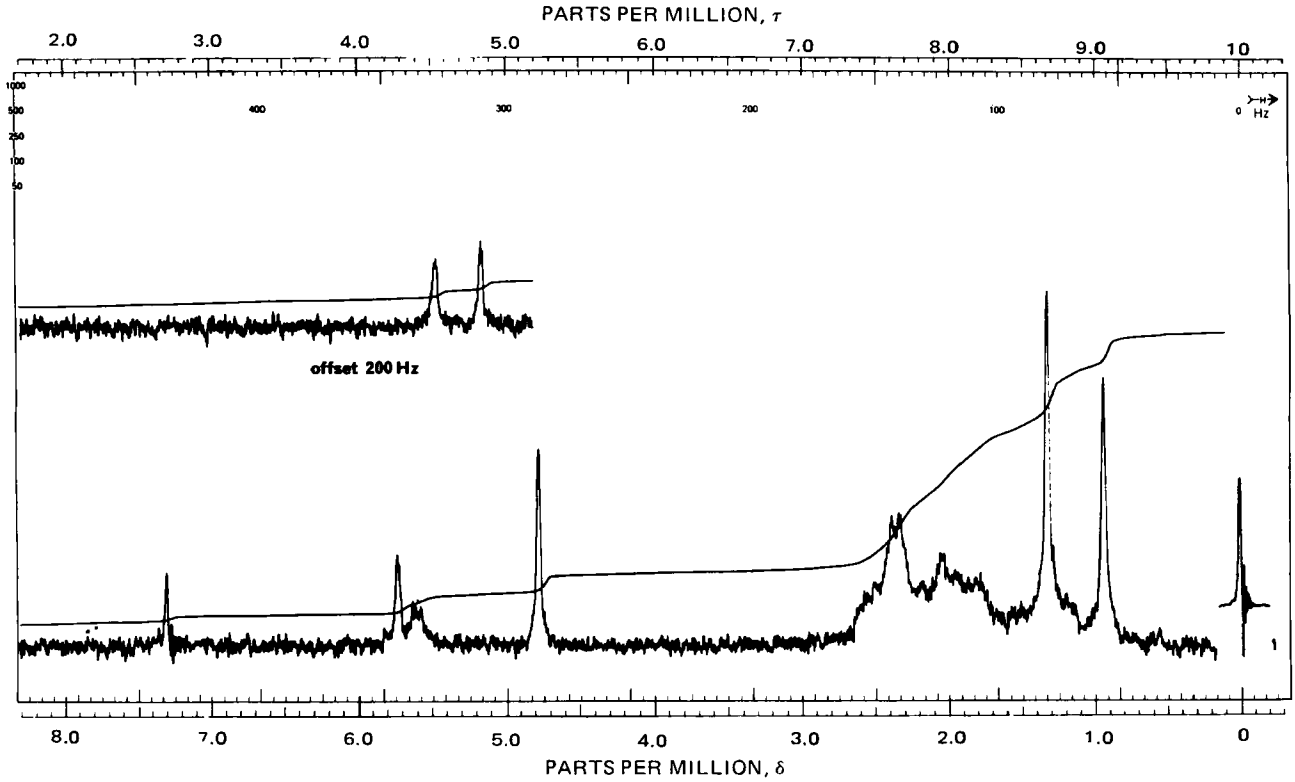
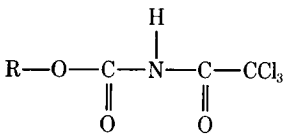


Figure 1—*The 60-MHz NMR spectrum of the trichloroacetyl isocyanate derivative of 11β,21-dihydroxypregn-4-ene-3,20-dione in deuterochloroform.*

are not sufficiently soluble in deuterochloroform for the determination of their NMR spectra.



type I

A note by Trehan *et al.* (7) led to a detailed study of the NMR spectroscopy of the trichloroacetyl carbamate derivatives of numerous corticosteroids and chemically related compounds of pharmaceutical interest. Polyhydroxy compounds should react at each hydroxyl group, with the carbamate proton signal in the NMR spectrum for each such hydroxyl appearing as a distinct singlet in a region of the spectrum isolated from all other signals.

EXPERIMENTAL

Approximately 40 mg of a hydroxysteroid[1] was accurately weighed into a 1-ml volumetric flask. Approximately 0.5 ml of deuterochloroform was added to the flask, followed by trichloroacetyl isocyanate[2] (~20 μl/hydroxyl group). Deuterochloroform was added to volume, and the flask was shaken until dissolution occurred.

The NMR spectrum was obtained[3] in the usual manner at 40 ± 2°, with tetramethylsilane added as the internal standard. Chemical shifts are reported as hertz downfield from tetramethylsilane (0 Hz).

[1] Steroids were obtained from the Pharmaceutical Chemistry Division collection of reference materials.

[2] Eastman Kodak Co., Rochester, N.Y.

[3] Varian A60A NMR spectrometer, Varian Associates, Georgetown, Ontario, Canada.

Table II—Concentration Effect on the Chemical Shift[a] (Hertz) of the Three Carbamate Proton Signals in the NMR Spectrum of the Reaction Product of Prednisolone with Trichloroacetyl Isocyanate

Origin of Proton	Concentration, *M*			
	0.07	0.14	0.21	0.28
11β	513	520	525	527
17α	551	566	572	575
21	538	548	554	556

[a] Chemical shifts downfield from tetramethylsilane (0 Hz).

Some samples (dexamethasone, dexamethasone acetate, paramethasone acetate, and triamcinolone diacetate) were left for 24 hr at room temperature after being made up to volume because of the very slow reaction to the severely hindered 17α-hydroxyl group with the reagent. Other slow reactions are noted in Table I.

The concentration dependence of the signal from the carbamate proton was determined by accurately weighing 10, 20, 30, and 40 mg of prednisolone and making up to 0.4 ml after adding 15, 30, 45, and 60 μl, respectively, of trichloroacetyl isocyanate.

Table III—Effect of Each 11β, 17α-, and 21-Hydroxyl Group Carbamate on the Chemical Shift[a] (Hertz) of the Carbamate Proton Signal of the Others

Compound	Hydroxyl		
	11β	17α	21
II	508	—	—
XI	510	—	529
VII	—	—	526
V	—	562	—
XII	—	594	547
XVIII	512	563	547
XIX	520	566	547

[a] Chemical shifts downfield from tetramethylsilane (0 Hz).

Table IV—Effect of Introducing an 11-Carbonyl Group on the Chemical Shift[a] (Hertz) of the Carbamate Proton Signals from 17α- and 21-Hydroxyl Groups

Compound	Hydroxyl	
	17α	21
XII	594	547
XIV	583	549
XIII	574	—
XV	563	—
V	562	—
VI	554	—
VII	—	526
VIII	—	528

[a] Chemical shifts downfield from tetramethylsilane (0 Hz).

RESULTS AND DISCUSSION

Chemical shifts are reported in Table I for the carbamate proton signals and also for the signals from the carbinol protons, the tertiary methyl protons (C-18 and C-19), and the vinylic protons. All spectra were obtained at a concentration of 40 mg/ml, because, as shown in Table II, there is a marked concentration effect on the position of the carbamate proton signals. A typical spectrum is shown in Fig. 1 (Compound X).

Table III shows the effect of each 11β-, 17α-, and 21-hydroxyl group on the chemical shift of the others (in terms of the respective carbamate NH signals). From Table III, the following conclusions may be drawn: (*a*) the chemical shifts of the 11β- and 21-hydroxyl group derivatives are independent of each other and (*b*) the 17α- and 21-hydroxyl group derivatives are mutually deshielding.

Introducing a 17α-hydroxyl into the parent molecule deshields the 21-carbamate proton signal by approximately 20 Hz; likewise, introducing a 21-hydroxyl deshields the 17α-carbamate proton signal by approximately 30 Hz. However, an 11β-hydroxyl counteracts the effect of the 21-hydroxyl on the 17α-carbamate proton signal (XII *versus* XVIII). Comparison of XVIII with XIX shows that the Δ^1-function has a minimal effect on the chemical shift of the 17α- and 21-carbamate NH signals but deshields the 11β-carbamate signal by 8 Hz.

Table IV shows the effects of introducing an 11-carbonyl group on the chemical shift of the 17α- and 21-carbamate proton signals. The compounds are grouped in pairs, with the 11-carbonyl derivative appearing below the corresponding unoxygenated compound. From these results, it can be seen that the 17α-carbamate proton signal is shielded by approximately 10 Hz, whereas the 21-carbamate proton signal is deshielded by only 2 Hz, which is not significant.

Table V shows the effect of acetylation of the 21-hydroxyl group on the chemical shift of the carbamate proton signals at 11β and 17α. Some general comments may be made about these results, noting that there is one significant exception (XXVIII/XXIX). The 17α-carbamate proton signal is shielded by approximately 20 Hz, and the 11β-carbamate proton signal is shielded by 5–7 Hz upon acetylation of the 21-hydroxyl.

Table V—Effect of 21-Acetylation on the Chemical Shift[a] (Hertz) of the 11- and 17-Carbamate Proton Signals

Compound	Proton		
	11β	17α	21
XII	—	594	547
XIII	—	574	—
XIV	—	583	549
XV	—	563	—
XVI	—	590	550
XVII	—	576	—
XIX	520	566	548
XX	513	547	—
XXI	522	606	545
XXII	517	586	—
X	510	—	529
XI	503	—	—
XXVIII	531	611	547
XXIX	529	576	—

[a] Chemical shifts downfield from tetramethylsilane (0 Hz).

Table VI—Predictions of Major Signals (Hertz) in the NMR Spectra of the Carbamates of XV and XX[a]

Compound	Protons	Predicted	Observed
XV	C-18 Methyl	48	46
	C-19 Methyl	85	86
	21	293	291
	C-4 Vinylic	344	344
	17α-Carbamate	563	563
XX	C-18 Methyl	63	63
	C-19 Methyl	78	78
	21	291	292
	11α	342	344
	C-4 Vinylic	361	363
	C-2 Vinylic	374	377
	C-1 Vinylic	424	424
	11β-Carbamate	520	513
	17α-Carbamate	545	547

[a] Chemical shifts downfield from tetramethylsilane (0 Hz).

Compound XXVIII has a 16α-methyl substituent, which sterically interacts with the 17α-hydroxyl group. The best evidence for this hindrance is the fact that XXVIII, together with XXIX, XXXIV, and XXXVI, all of which possess 16α-substituents, requires 24 hr for the 17α-carbamate derivative to form. The distortion created by the 16α-substituent in XXVIII has the effect that, when a 21-acetyl group is introduced (XXIX), the 17α-carbamate proton signal is shielded by 35 Hz, whereas the shielding of the 11α-carbamate proton signal is reduced to 2 Hz.

For two of the compounds studied, XV and XX, predictions were made, based on accumulated results, of the major signals in their NMR spectra. These results are shown in Table VI. Some signals, such as the vinylic protons, are easy to predict. The tertiary methyl signals are also relatively easy to predict since tables are available to assist in the prediction (11). As more compounds are studied, further rules may be discovered to assist in more accurate predictions of the carbamate proton signals.

The reverse situation is immediately obvious: that given such an NMR spectrum, one can deduce the structure and stereochemistry of functional groups. Therefore, the technique is of value in the structure determination in unknown compounds, thereby extending its analytical applications.

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