in situ REACTIONS WITH TRICHLOROACETYL ISOCYANATE AND THEIR APPLICATION TO STRUCTURAL ASSIGNMENT OF HYDROXY COMPOUNDS BY ^{1}H-NMR SPECTROSCOPY. A GENERAL COMMENT

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The application possibilities of the *in situ* reactions of trichloroacetyl isocyanate (TAI method) to structural assignment of alcohols by means of ^{1}H-NMR spectroscopy are critically evaluated. The possibilities of TAI acylations in various solvents are demonstrated and the reactivity of various functional groups, potentially complicating or extending the applications of TAI, is discussed. In addition to their use in structural analysis such reactions also can serve as model experiments for preparative purposes.

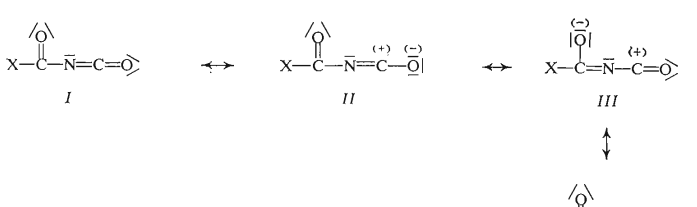
The performance of the *in situ* reactions in the sample tube of the NMR spectrometer represents both the optimum utilization of NMR spectroscopy for the study of chemical reactions and for an efficient exploitation of a chemical reaction for structural assignment of NMR spectra. In principle all chemical reactions can be studied in this manner which take place in a homogeneous liquid or gas phase. A purposeful performance of the studies with structurally known reactants represents an important application field of NMR spectroscopy. However, an exploitation of the *in situ* reactions for structurally analytical purposes is less common. In this case the reaction of the investigated substance is carried out with a known reagent. The structure of the starting compound and of the product of the *in situ* reaction is substituted by the NMR spectrum. The induced, characteristic changes of the spectrum usually enable the structure identification not only of the reaction centre, but of its closest proximity as well. The amount of the information on the structure obtained is proportional to the total change of the chemical shifts and the coupling constants. A suitably chosen, chemically induced non-linear transformation of the distribution of the chemical shifts of the starting compound may possess, in principle, the same informative value as the linear expansion of the spectrum by the increase of the intensity of the external magnetic field. In this sense the *in situ* reactions appears as a very useful and non-expensive extension of the NMR technique.

The structure determination of alcohols is a classical structural problem that has most strongly stimulated the application of the *in situ* reactions in the NMR sample tube. It consists of three parts: *1*) determination of the number of OH-groups, *2*) classification of the OH-groups into primary, secondary, and tertiary (determination of the number of α-CH), and *3*) determination of the character of the substituents of the C_α-atom and the relative configuration of the OH-group. This problem is solvable in principle by means of ^{1}H-NMR spectroscopy by direct identification of the corresponding spin systems, with the assignment of the OH-signals by decoupling, exchange experiments, or by varying the concentration, temperature or solvents. However, these procedures are suitable only if it is possible to decrease the mobility of the OH-protons so that their vicinal

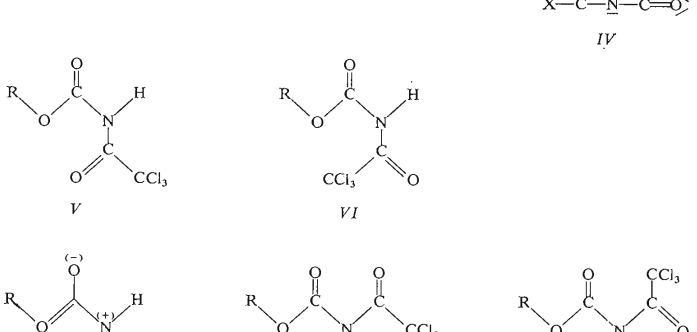
or long-range interactions may be observed. Often these procedures fail, either owing to the absence of structurally defined OH-signals or to the unsufficient selectivity of the α- or β-shifts with respect to OH (for example in the application of the lanthanide shift reagents.) In such instances ^{1}H-NMR spectroscopy makes use of preparative transformations of the OH-groups to the more easily defined OR-groups (for example acetylation and benzoylation[1], dichloroacetylation[2], formylation[3] or trifluoroacetylation[4] in combination with ^{19}F-NMR (ref.[5]), methylation[6] or trimethylsilylation in combination with ^{29}Si-NMR (ref.[7]), or acylation with optically active acids[8]). After these conversions structurally characteristic acylation or alkylation shifts of the CH-signals *i.e.* (α, β, γ, ...) or of the signals of the acyl or alkyl groups are usually observed. Among other conversions of alcohols, combined with ^{1}H-NMR measurements, the eliminations to exo- or endocyclic double bonds (for example ref.[9]) were used. All these preparative procedures require a relatively large amount of substance, and in a number of polyfunctional cases they can lead to the formation of products the relation of which to the starting substance is not evident. They also often fail owing to the reactivity of the hydroxy groups. In the general case of a hydroxy derivative with an unknown structure and small amount of substance (less than 10 mg) the preparative methods cannot be applied. In principle all these disadvantages are eliminated by *in situ* acylations. Goodlett[10] investigated the possibility of *in situ* acylations with diphenylketene, phenyl isocyanate, and trichloroacetyl isocyanate, and he found that trichloroacetyl isocyanate (TAI) possesses all the properties necessary for an efficient universal acylation reagent. When TAI is applied to alcohols the OH protons are converted to *a priori* less mobile imide NH protons, with chemical shifts in the region of $\delta > 8$, where they are relatively easily accessible for quantitative measurements. TAI-acylation induces characteristic acylation shifts, similar to acetylation, but no group is introduced which would complicate the ^{1}H-NMR spectrum. TAI is very reactive and mostly it reacts rapidly even with sterically hindered tertiary hydroxy groups[10].

In spite of the considerable attractiveness of the TAI method it was not widespread at first, and its use was limited for a long time to sporadic structural analytical applications to hydroxy compounds[11-27] or thiols[28]. In recent years its use has been expanded even to acylations of amines[29,30] in ^{1}H-NMR, as well as in ^{13}C-NMR (ref.[29]). The cycloadditions of TAI to enol ethers[31] have also been investigated. In our laboratory the TAI method has been applied for a number of years, mainly for the structure elucidation of natural hydroxy compounds[9,32-48]. We have also investigated the reactions of TAI with various functional groups as they are known from preparative chemistry of acyl isocyanates, and also their possible *in situ* adaptations, both for the purposes of ^{1}H-NMR spectra assignments, and for the modelling of reaction conditions for preparative utilization. The purpose of our communication is the presentation of a general view both on the problems of *in situ* application of TAI generally, and also on the present use of TAI for the structural assignment of alcohols.

The reactions of acyl isocyanates are based on their electronic structure, represented by the structures *I*–*IV* (ref.[49]).



I *II* *III* *IV*



V *VI* *Va* *VII* *VIII*

The first type is 1,2-addition, taking place analogously as in isocyanates[50] *via* an attack of the nucleophilic centre of the substrate on the electrophilic C atom of the NCO group (contributions of *II* (major) and *IV* (minor[51])). The second type, proper to acyl isocyanates, is 1,4-cycloaddition *via* the structure *III* (ref.[49]). The reactivity of X-CONCO increases with the electronegativity of X, and for TAI – for example – relation C_6H_5—CO—NCO < CCl_3CO—NCO < CF_3CO—NCO (ref.[52]) applies.

Table I gives a review of some main types of reactions of acyl isocyanates that can be observed either as main reactions or co-reactions in *in situ* applications of

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[a] Papers in which the appropriate reaction is described as "*in situ* in the NMR sample-tube' are italicized. [b] This paper. [c] The structures of the adducts are proposed on the basis of analogous reactions of sulfonyl isocyanates (ref.[69,70]). [d] The elimination took place for both configurations of the OH group for both types of annelations of the homobicyclic system.

TABLE I
List of Reactions of Acyl Isocyanates (X—CO—N=C=O)

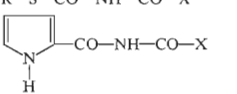
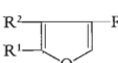
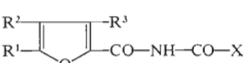
No.	Substrate	Product	Ref.[a]
	1,2-Addition reactions with R—Y—H (Y = O, N, S, C, Cl)		
1	R—OH	R—O—CO—NH—CO—X	9–27, 29, 32–48, 49
2	$R^1R^2C{=}N{-}OH$	$R^1R^2C{=}N{-}O{-}CO{-}NH{-}CO{-}X$ (R^2 = R, H or NH_2)	49, 53
3	R—CO—OH	R—CO—O—CO—NH—CO—X + R—CO—O—CO—R	49[b]
4	R^1R^2NH	$R^1R^2N{-}CO{-}NH{-}CO{-}X$ (R^2 = R or H)	29, 49, 53
5	$R^1{-}O{-}NH{-}R^2$	$R^1{-}O{-}NR^2{-}CO{-}NH{-}CO{-}X$ (R^2 = R or H)	49, 53
6	$R^1{-}NH{-}NH{-}R^2$	X—CO—NH—CO—NR^1—NR^2—CO—NH—CO—X (R^1, R^2 = R or H)	49, 53
7	$R^1R^2C{=}N{-}NH{-}R^3$	$R^1R^2C{=}N{-}NR^3{-}CO{-}NH{-}CO{-}X$ (R^2, R^3 = R or H)	49, 53
8	$R^1{-}CO{-}NH{-}R^2$	$R^1{-}CO{-}NR^2{-}CO{-}NH{-}CO{-}X$ (R^2 = R or H)	49, 53
9	$R^1{-}SO_2{-}NH{-}R^2$	$R^1{-}SO_2{-}NR^2{-}CO{-}NH{-}CO{-}X$ (R^2 = R or H)	49, 53
10	$R^1{-}CS{-}NH{-}R^2$	$R^1{-}CS{-}NR^2{-}CO{-}NH{-}CO{-}X$ (R^2 = R or H)	49, 53
11	$R^1{-}O{-}CO{-}NH{-}R^2$	$R^1{-}O{-}CO{-}NR^2{-}CO{-}NH{-}CO{-}X$ (R^2 = R or H)	49, 53
12	$R{-}NH{-}CO{-}NH_2$	R—NH—CO—NH—CO—NH—CO—X	49, 53
13	$R{-}NH{-}CS{-}NH_2$	R—NH—CS—NH—CO—NH—CO—X	49, 53
14	R—SH	R—S—CO—NH—CO—X	28, 49
15			36
16			36
17	HCl	Cl—CO—NH—CO—X	49[b]
	1,2-Addition reactions with elimination of carbon dioxide		
18	H_2O	$X{-}CO{-}NH_2$	49[b]
19	R^1R^2SO	$R^1R^2S{=}N{-}CO{-}X$	49[b]
20	$R^1R^2N{-}CO{-}R^3$	$R^1R^2N{=}C{-}NR^3{-}CO{-}X$	49[b]

TABLE I
(*Continued*)

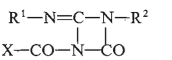
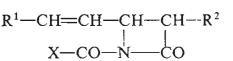
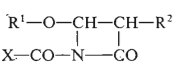
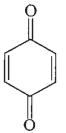
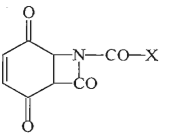
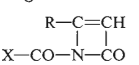
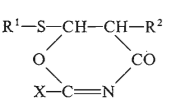
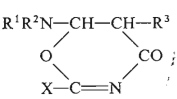
No.	Substrate	Product	Ref.[a]
	1,2- and 1,4-Cycloaddition reactions		
21	$R^1-N=C=N-R^2$		54, 55
22	$R^1-CH=CH-CH=CH-R^2$		56, 57
23	$R^1-O-CH=CH-R^2$		*31*, 52
24			58
25	$R-C\equiv H$		59, 60
26	$R^1-S-CH=CH-R^2$		61
27	$R^1R^2N-CH=CH-R^3$		62

TABLE I
(*Continued*)

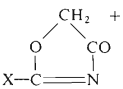
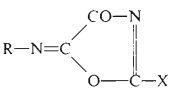
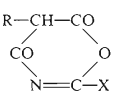
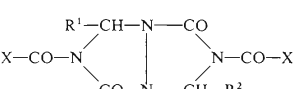
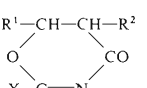
No.	Substrate	Product	Ref.[a]
28	CH_2N_2		63, 64
29	R—N≡C		53, 54, 65, 66
30	R—CH=C=O		67
31	R^1—CH=N—N=CH—R^2		68
32	R^1—CH=CH—R^2		52, 59

TABLE I
(*Continued*)

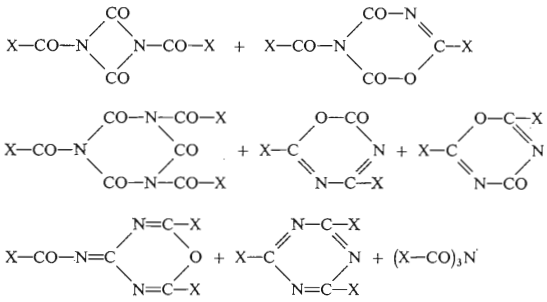
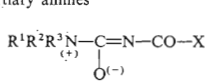
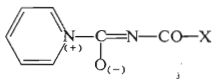
No.	Substrate	Product	Ref.[a]
	Dimerization and trimerization of acyl isocyanates		
33	X—CO—N=C=O		49, 68
34	$R^1R^2R^3N$		69, 70, 53[c]
35			69, 70, 53[c]

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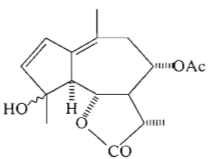
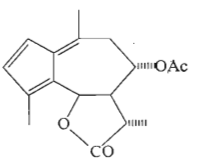
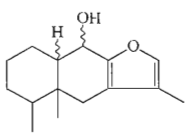
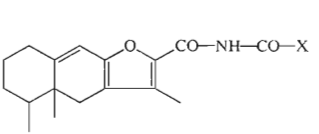
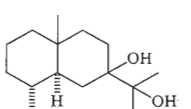
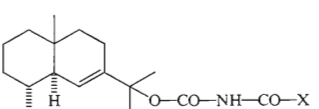
TABLE I
(*Continued*)

No.	Substrate	Product	Ref.[a]
	Elimination reactions		
36			53
37			53
38			53

TAI. The first group consists of 1,2-addition reactions with substances of R-Y-H type (Y = O, N, S, C, Cl, entries *1–17*), or typical acylation reactions. These reactions are always indicated by NH-signals in the $\delta = 8-13$ ppm region and changes of the NMR spectrum of the substrate, that can be interpreted on the basis of the acylation effects. The second group consists of 1,2-additions accompanied by CO_2 elimination (entries *18–20*). The reactions of this type can be observed, for example, as co-reactions on TAI acylations in the presence of water, or as reactions of the employed solvents, for example dimethyl sulfoxide, (entry *19*) or dimethylformamide (entry *20*). These reactions are indicated by the changes of the NMR spectrum of the substrate only, and the NH-signals only appear in the case of water which gives trichloroacetamide with TAI (NH signals in the region $\delta = 6-8$ ppm) which reacts further slowly as in the reaction of type *18* to N,N'-bistrichloroacetylurea (NH in the region $\delta = 11-12$ ppm). The third group consists of 1,2-cycloadditions (entries *22–25*) or 1,4-cycloadditions (entries *26–32*) to activated double or triple bond. Contrary to acylations in these reactions no NH-signals appear and no elimination of CO_2 takes place, but only such changes of the spectrum of the substrate as are considerably different from the acylation effects ($sp^2 \rightarrow sp^3$, $sp \rightarrow sp^2$). Typical co-reactions are dimerization and trimerization of acyl isocyanates (entry*33*). In the TAI method the products of these reactions are not detectable in ^{1}H-NMR spectra, and unless such substrates are used which catalyze these reactions their concentration is very low and their presence has no effect on the spectrum of the substrate. A further group consists of the addition reactions of acyl isocyanates with tertiary amines in which only the free electron pair of the N-atom participates. In the spectrum this reaction produces down-field shifts of the signals of protons of the substrate, bound in the neighbourhood of N-atoms, similarly as in N-acylation reactions; however, in contrast to acylations the formation of NH-signals does not take place. This reaction should be taken into account, for example, in the applications of the TAI method to alkaloids. The last group consists of elimination reactions of hydroxyls leading to characteristic change of the NMR spectrum of the substrate corresponding to the introduction of the double bond ($sp^3 \rightarrow sp^2$).

The mentioned features mostly permit an estimation of the kind of the reaction taking place in the analysed solution and an adequate interpretation of the observed changes of the NMR spectrum. A trivial complication of the TAI method is always the reaction with water (from the sample, the solvent, the air) under formation of trichloroacetamide. The latter, of course, does not upset the interpretation of the NMR spectrum directly, but indirectly complications may arise in cases when the concentration of water in the reaction mixture is high and the main reaction of the substrate is slow. In such instances both the consumption of TAI is high and also crystallization of trichloacetamide may take place, or also its NH_2-signal may begin to interfere. Therefore it is advisable to use as dry solvents as possible. Traces of mineral acids do not interfere; for example HCl which is usually present in deuterio-

chloroform also reacts with TAI under formation of trichloroacetylcarbamoyl chloride. On the contrary, this co-reaction is very useful, because it completely deprotonizes the reaction medium. This may be sometime useful in TAI applications to substances sensitive to traces of acids. Further potential complications are the reactions of TAI with solvents. This complication is met when the starting substrate or its reaction product with TAI are insoluble in common unreactive solvents and therefore a solvent must be selected that does react with TAI. In connection with this problem we tested the reactivity of a series of solvents commonly used in NMR spectroscopy with TAI and also investigated the relative reactivity of TAI with substrates and solvents in model experiments with a mixture of primary, secondary and tertiary alcohols (see Experimental). A review of the results of these experiments is given in Table II. The large majority of the solvents does not react with TAI, and their addition does not change the chemical shifts of proton signals of the solvent significantly. The same holds for proton signals of unreactive substrates ($\Delta\delta \leqq \pm 0{\cdot}01$). Among the reactions with reactive solvents those with dimethyl sulfoxide to dimethylsulfamidine (Table I, *19*) and with dimethylformamide to dimethylformamidins (Table I, *20*) are important, that take place under generation of CO_2. These reactione take place, especially in the case of dimethyl sulfoxide, relatively slowly in comparison with the reaction of the OH groups, so that the reactions with more reactive groups can be carried out in these solvents. Trifluoroacetic anhydride itself is a very reactive *in situ* acylation reagent. In this solvent alcohols are converted to trifluoroacetates that do not further react with TAI. Trifluoroacetic acid acylates OH more slowly than TAI, but as TAI forms with it the more reactive anhydride (Table I, *3*) the consequence of its addition is merely an acceleration and completion of trifluoroacetylation. In contrast to this acetic acid and acetic anhydride do not acylate *in situ* during the standard procedure (see Experimental) and therefore they may be used for TAI acylations. The behaviour of acetic acid and also HCl enables another practical nuance of the *in situ* applications of TAI, *i.e.* the possibility of carrying it out after the exchange experiment, for example with OH or NH groups. It is suitable to use traces of non-deuterated acetic acid for the exchange experiment, which preserves the observability of the NH signals in the $\delta = 8-13$ ppm region.

The main complication consists in the relatively large reactivity range of acyl isocyanates. However, some reactions require a long reaction time or heating, and under standard conditions they do not manifest themselves at a detectable level. For example the reaction of the double bond of norbornene with TAI in dichloromethane at 20°C is complete after 12 days[52]. However, it is necessary to reckon with these reactions as with potential complications of the *in situ* applications of TAI, especially if the time of the experiment is long. According to our present experience a number of common functional derivatives, as for example ketones, anhydrides, esters, aldehydes, lactones, dienes, olefins, epoxides, *etc.* appear as unreactive under standard procedure.

TABLE II

TAI-Acylation of Alcohols in Various Solvents

Solvent[a]	Re-activity[b]	δ-Values after addition of TAI					NH[c]	TAI-induced acylation shifts[d]				
		EtOH		i-PrOH		t-BuOH						
		CH_3	CH_2	CH_3	CH	CH_3		CH_3	CH_2	CH_3	CH	CH_3
Cyclohexane	(−)	[e]	4·23	[e]	5·00	[e]	8·67; 8·60; 8·55	[e]	0·71	[e]	1·16	[e]
[$^2H_6$]-Benzene	(−)	0·81	3·77	0·90	4·74	1·20	7·96 (3)	−0·03	0·55	0·05	1·16	0·25
[$^2H_3$]-Toluene	(−)	0·91	3·85	0·98	4·76	1·26	8·31 (3)	−0·03	0·51	0·05	1·10	0·25
[$^2H_5$]-Nitrobenzene	(−)	1·16	4·19	1·18	5·00	1·44	9·03 (2); 8·98	0·02	0·54	0·06	1·04	0·24
[$^2H_3$]-Nitromethane	(−)	1·23	4·19	1·22	4·94	1·43	8·87 (3)	0·18	0·67	0·19	1·10	0·32
Dichloromethane	(−)	1·27	4·24	1·26	5·01	1·46	8·43 (3)	0·16	0·66	0·18	1·11	0·31
Deuteriochloroform	(−)	1·30	4·28	1·28	5·03	1·48	8·54 (3)	0·14	0·65	0·15	1·09	0·29
Tetrachloromethane	(−)	1·38	4·30	1·36	5·04	1·55	9·16; 9·03; 8·82	0·22	0·71	0·23	1·13	0·36
1,2-Dichloroethane	(−)	1·26	4·22	1·25	4·97	1·45	8·44 (3)	0·16	0·67	0·17	1·11	0·31
Hexachlorobutadiene	(−)	1·27	4·23	1·26	4·99	1·45	8·60; 8·52; 8·44	0·18	0·70	0·20	1·14	0·33
Tetrahydrofuran	(−)	1·22	4·24	1·22	4·95	1·44	10·25 (3)	0·19	[e]	0·21	[e]	0·36
Dioxane	(−)	1·25	[e]	1·25	4·97	1·46	9·83; 9·69; 9·64	0·19	[e]	0·22	[e]	0·35
[$^2H_6$]-Acetone	(−)	1·21	4·18	1·21	4·93	1·42	10·15; 9·99, 9·75	0·17	0·68	0·19	1·10	0·31
[$^2H_6$]-Dimethyl sulphoxide	(+)[f]	1·24	4·18	1·25	4·92	1·45	11·54 (3)	0·24	0·78	0·27	1·20	0·40
[$^2H_3$]-Acetonitrile	(−)	1·23	4·18	1·23	4·94	1·44	9·21 (3)	0·18	0·70	0·21	1·13	0·34
Ethyl acetate	(−)	1·25	4·21	1·24	4·97	1·46	9·77 (2); 9·43	[e]	0·71	[e]	[e]	[e]
[$^2H_5$]-Pyridine	(+)[g]	[g]	[g]	[g]	[g]	[g]		[g]	[g]	[g]	[g]	[g]
N,N-Dimethylformamide	(+)[h]	1·21	4·17	1·21	4·91	1·42	[g]	0·17	0·69	0·19	1·05	0·32
Hexamethylphosphoricamide	(−)	1·22	4·16	1·22	4·92	1·43	[e]	0·19	0·73	0·21	[e]	0·34
Acetic acid	(+)[i]	1·24	4·23	1·24	5·00	1·45	[e]	0·13	0·60	0·14	1·02	0·27
Acetic anhydride	(−)	1·22	4·20	1·22	4·96	1·43	[e]	0·13	0·61	0·14	1·04	0·27
Trifluoroacetic acid	(+)[j]	[j]	[j]	[j]	[j]	[j]	[j]	[j]	[j]	[j]	[j]	[j]
Trifluoroacetic anhydride	(−)	[k]	[k]	[k]	[k]	[k]	[k]	[k]	[k]	[k]	[k]	[k]

TAI experiments with compounds containing a single reactive group are always simple, but those containing several reactive groups, either structurally equal or different, are more difficult. In the case of polyfunctional substances with groups of various reactivity the *in situ* applications of TAI may be approached, in principle, with the known philosophy of the "protecting groups", and it is possible to combine a number of *in situ* reactions with reagents having different reactivity, enabling the blocking of some of the group against a TAI attack. In substances with comparably reactive groups the use of TAI might produce a mixture of partially acylated derivatives, the spectra of which can be analysed directly in some instances. In the case of groups with a very different reactivity their stepwise reaction with TAI may also be observed. According to our experiences TAI may also be combined with lanthanide shift reagents.

Of course, it is evident that the application of TAI is always connected with a certain risk of the destruction of the substrate. If only a small amount of the substance is available, it is advisable to carry out first a separate test with TAI using an NMR or a TLC detection. Such orienting tests can be carried out especially simply in ^{1}H-FT-NMR spectroscopy.

[a]Measured on Varian HA-100, with hexamethyldisiloxane as internal standard: a mixture of ethanol, 2-propanol and tert-butanol in a 3 : 2 : 1 molar ratio and the standard experimental procedure were applied. [b] The reactivity of the solvents was tested separately by the standard procedure in deuteriochloroform solution; (+) means that the solvent does react with TAI, while (–) means that it does not. [c] If the NH signals are resolved, their order is ethanol, 2-propanol, tert-butanol. [d] TAI-induced acylation shifts are defined according to equation (*2*). [e] The signals are overlapped by the signals of the solvent; TAI-acylation shifts were not obtained. [f] Dimethyl sulfoxide reacts with TAI according to the type II. *10*. (Table I), the product is $(CH_3)_2S{=}N{-}COCCl_3$ ($\delta_{CH_3} = 2{\cdot}84$). The reaction is substantially slower than the acylation of alcohol. Water present in the solvent gives trichloroacetamide with TAI (type II.*18*, Table II), with $\delta_{NH} = 8{\cdot}00$ ppm, which reacts slowly with TAI to N,N′-bis(trichloroacetyl)urea ($\delta_{NH} = 11{\cdot}60$ ppm). [g] Pyridine reacts with TAI immediately under formation of a precipitate; it cannot be used as solvent for TAI-acylation of alcohols. When TAI reacts with pyridine in deuteriochloroform the spectrum of the product $C_5H_5N{-}CONCO{-}CCl_3$ is obtained (Table II V.*35*), with $\delta_{\alpha-H} = 8{\cdot}87$, $\delta_{\beta-H} = 7{\cdot}46$ and $\delta_{\gamma-H} = 7{\cdot}91$. — [h] Dimethylformamide reacts with TAI more slowly than alcohols, according to type II.*20* (Table II), under formation of $(CH_3)_2N{-}{-}CH{=}N{-}CO{-}CCl_3$ with $\delta_{CH_3} = 2{\cdot}72$ and $2{\cdot}88$, $\delta_{CH} = 7{\cdot}93$. [i] Acetic acid reacts with TAI more slowly than alcohols, according to type I.*3* (Table I) under formation of $(CH_3CO)_2O$ with $\delta_{CH_3} = 2{\cdot}21$ and $CH_3{-}COOCONHCOCCl_3$ with $\delta_{CH_3} = 2{\cdot}34$ and $\delta_{NH} = 11{\cdot}45$. [j] Trifluoroacetic acid reacts with TAI probably according to type I.*3* (Table I) under formation of $(CF_3CO)_2O$ and $CF_3COOCONHCOCCl_3$ which cannot be detected in the ^{1}H-NMR spectrum. Trifluoroacetic acid itself reacts with the tested alcohols and converts them to trifluoroacetates within one hour. Addition of TAI enhances trifluoroacetylation through the formation of the more reactive trifluoroacetic anhydride. The solvent cannot be used to obtain TAI-induced acylation shifts of alcohols. [k] Trifluoroacetic anhydride reacts with alcohols under formation of trifluoroacetates which do not further react with TAI. TAI-induced acylation shifts cannot be obtained.

APPLICATION OF THE TAI-METHOD IN STRUCTURAL STUDIES OF HYDROXY DERIVATIVES

The main reaction of TAI with hydroxy derivatives is the reaction of the type *I* in Table I, the main products of which are trichloroacetylcarbamoyl derivatives R—O—CO—NH—CO—CCl_3 (O-TAC derivatives). In a number of heteropolyfunctional derivatives TAI-reactions can take place at a similar rate with groups other than OH, as for example N, S, C-acylations (entries *1–6*, Table I). The reactivity of alcohols with TAI is usually very high and reactions with an excess of TAI are usually terminated before the spectrum can be recorded[10]. This is true, however, of primary and secondary hydroxy groups only. In the case of tertiary OH groups the reactivity with TAI is considerably dependent on steric effects[12,29]. In a number of cases the tertiary OH group reacted completely only after several hours or even days of standing (ref.[21,38,39]).The phenolic group also belongs among less reactive groups, evidently owing to the delocalization of the free electron pair of the oxygen atom and the stabilization of its trigonal configuration, which is unfavourable for the attack with TAI. Steric effects play a lesser role and the reactions of TAI with, for example, 2,4,6-tri-tert-butylphenol and *p*-nitrophenol take place approximately equally slowly. On the other hand 2,6-dimethoxyphenol reacts relatively rapidly, equally as phenol or *p*-cresol.

We tested the relative reactivity of the alcoholic and phenolic OH groups in a "titration" experiment, carried out with equimolar mixtures of ethanol, 2-propanol, tert-butanol and *p*-cresol in deuteriochloroform. The percentual fractions of the reacted substances plotted against the number of the added drops of TAI are shown in Fig. 1 (the invariance of the spectrum was tested before each subsequent addition). The reactivity of the OH groups decreases in the following order: prim- > sec- > tert- > phenol. This differing reactivity is useful for the study of polyhydroxy compounds. In such cases partial acylations may be observed during the titration

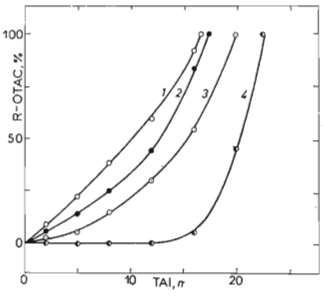


FIG. 1

Titration TAI Experiment with an Equimolar Mixture of Ethanol (*1*), 2-Propanol (*2*), tert-Butanol (*3*), *p*-Cresol (*4*)

The graph represents the dependence of the percentual part of the TAI derivative (R--OTAC) on the number of drops (*n*) of TAI.

experiment, in dependence on the relative reactivity of individual OH groups. In the case when the relative reactivity of the OH groups is similar a superposition of the initial spectrum can be observed and of the spectra of all possible partial TAC-derivatives and the totally acylated substance. If indicating the total number of the OH groups with n and the number of TAC-groups of an m-times acylated derivative with m, then – in a general non-symmetric case – the maximum number of the superimposed spectra, N, is given by

$$N = \sum_{m=0}^{n} \binom{n}{m} = 2^n , \qquad (I)$$

where the number $\binom{n}{m}$ means the number of the isomeric m-times acylated derivatives.

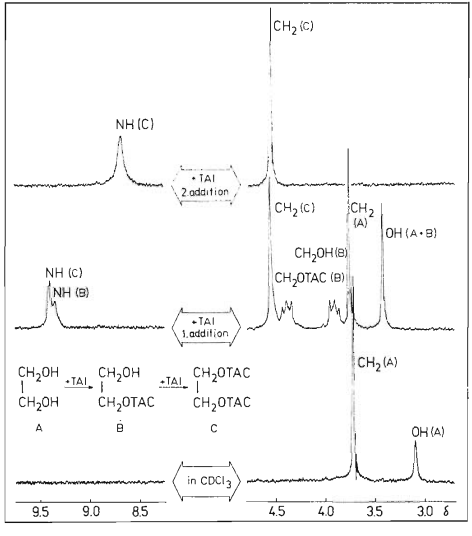


FIG. 2

Course of TAI Acylation of Ethylene Glycol

A case of partial acylation of two OH-groups of equal reactivity.

If the reactivity is different then the intensities of individual spectra also differ and some of the OH groups can be "titrated out" with TAI. In the first case the ^{1}H-NMR spectra are usually very complex and the difficulty of identification of individual components of the mixture vary from case to case. Fig. 2 shows the course of the experiment with ethylene glycol titrated with TAI. In this case first the superposition of three spectra can be observed, *i.e.* the spectrum of the starting glycol, of the non-symmetric mono-TAC derivative (AA'BB') system and the symmetric di-TAC derivative. With an excess of TAI only the spectrum of the di-TAC derivative results. A case is demonstrated in Fig. 3 where the reactivity of two tertiary OH-groups is different. It is the case of a compound[71] containing two non-equivalent geminal dimethyl groups $(CH_3)_2C—OH$, affording four singlets in the ^{1}H-NMR spectrum. The problem consists in the assignment of the corresponding pair of signals of the tert-methyl groups to individual geminal dimethyl groups. The solution can be carried out by means of partial acylation with TAI, assuming that the signals of the methyl groups belonging to nonacylated $(CH_3)_2C—OH$ groups in two different mono-TAC derivatives (B and C, Fig. 3) – which can be observed in this case – have similar chemical shifts as in the starting spectrum. In Fig. 3 it is evident that the signals at $\delta = 0{\cdot}97$ and $1{\cdot}25$ ppm

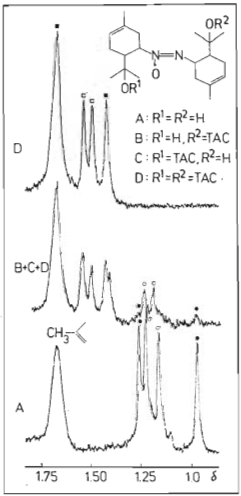


FIG 3.

Application of Partial Acylation with TAI for the Assignment of the Signals of Protons of Two Geminal Dimethyl Groups of Type $(CH_3)_2—C—OH$

A case of partial acylation of two OH-groups with unequal reactivity. The signals of non-acylated groups $(CH_3)_2C—OH$ are indicated with circles and the signals of acylated groups $(CH_3)_2C—OTAC$ with squares.

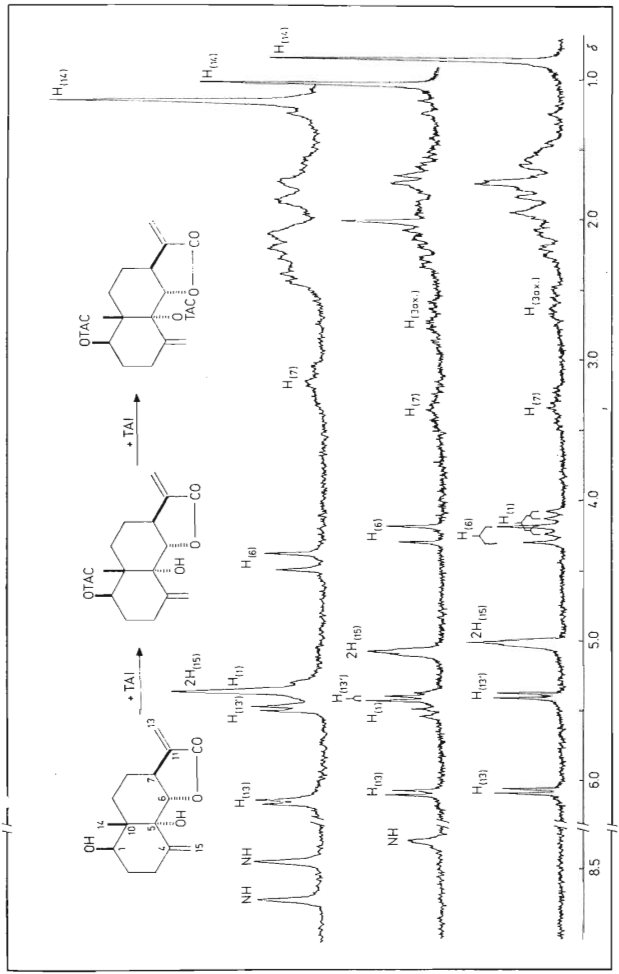


FIG. 4

A Case of the Observation of Stepwise Acylation of a Diol During a Standard TAI Experiment

The bottom trace shows the starting spectrum; the medium trace shows the partial acylation of the sec. OH (obtained after 10 minutes); the upper trace shows the complete acylation of both OH groups (after 2 days standing of the reaction mixture).

disappear rapidly after partial acylation, while the signals at $\delta = 1{\cdot}16$ and $1{\cdot}22$ ppm still remain relatively intensive. The signals of the methyls of the acylated groups are all shifted clearly downfield. With excess TAI again only four signals of the tertiary methyl groups ($\delta = 1{\cdot}41$, $1{\cdot}49$, $1{\cdot}53$, $1{\cdot}65$ ppm) of the di-TAC derivative result (Fig. 3*c*). This observation indicates the different reactivity of the tertiary OH group and the clear prevalence of one of the mono-TAC derivatives. Owing to the different concentration of mono-TAC derivatives the assignment of the pairs of signals of the tertiary methyl groups is evident. If the reactivity is very different, then a stepwise acylation can take place.

Fig. 4 shows the course of a TAI experiment (with an excess of TAI) with the sesquiterpenic dihydroxy lactone tanacetin[39]. The application of TAI first leads to an instantaneous acylation of the sec-hydroxyl on $C_{(1)}$, while the tertiary OH is not acylated significantly (1 NH signal), so that we can first observe and analyse the spectrum of the pure mono-TAC derivative with all the characteristic changes in the vicinity of $C_{(1)}$. After the analysis of the spectrum and in view of the low reactivity of the tertiary OH group the reaction mixture was allowed to stand in the sample tube at room temperature for 2 days. A new measurement already demonstrated the complete acylation even of the tertiary OH group (2 NH signals) with characteristic changes of the signals of the spectrum, belonging to the protons from the neighbourhood of $C_{(5)}$—OH (*cf.* ref.[39]).

According to our long-term tests the OTAC derivatives no longer react *via* NH with TAI (unless reacting in some other manner), and the transformation of OH to imide NH can be utilized for quantitative determination of OH groups *via* the NH signals, as was proposed originally[10,12]. The non-reactivity of the imide NH in the TAC group is probably a general property of the TAC groups, and we have observed it in all other TAC derivatives of the X-TAC type[53] (X=C, N, S). It is probably a consequence of the delocalization of the free electron pair of the imide N-atom. We have found that common imides, such as for example succinimide *etc.*, also do not react with TAI under the given conditions.

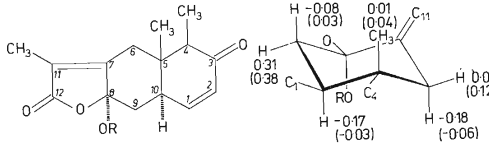
The signals of the NH-protons of the OTAC groups usually occur in the range of $\delta = 8-11$ ppm. Their positions are considerably dependent on concentration, and on dilution they are shifted upfield, as shown in Fig. 2 (this does not apply generally for X-TAC derivatives[53]). As shown in Table II the signals of NH appear in more polar solvents at a lower field ($\delta = 9-11$ ppm) than in the case of less polar ones ($\delta = 8-9$ ppm). The lowest δ-values of NH, observed in benzene, toluene and nitrobenzene are caused evidently by the ring-current effect in solvated TAC groups. Summarizing it can be stated that the positions of the NH signals of the OTAC groups are structurally insufficiently defined, and their exploitation for structure analysis — such as was tested in some studies[21] — is very problematic even under strictly analytical conditions.

The main structurally analytical criterion for hydroxy derivatives in the TAI method are the acylation shifts. They are known from acylation studies, especially for the acetyl group. In addition to the traditional classification to α-, β-, γ-acylation shifts (...C_γ—C_β—C_α—OH) in our practice the use of the definition of the acylation shifts enriched by the indexation of the position in which acylation took place, and the position of the proton the signal of which was affected, was found convenient, *i.e.*

$$\Delta^{(i)}\,\delta H_j(R) = \delta H_j(C_{(i)}\text{—}OR) - \delta H_j(C_{(i)}\text{—}OH)\,, \qquad (2)$$

where i indicates the position of the OH group and j the position of the affected proton in arbitrary numbering of the molecule, and R the acyl group. A positive $\Delta\delta$-value means the down-field and a negative value the up-field shifts, in accordance with the δ-scale.* In the α, β, γ-nomenclature the acylation shifts can then be indicated as Δ^α, Δ^β or Δ^γ, or in the case of phenols as $\Delta^{(o)}$, $\Delta^{(m)}$ or $\Delta^{(p)}$. According to our experience, when dilute solution (about 10 mg/0·3 ml) are used and strict analytical criteria are absent, a chemical shift of a value larger then ±0·05 ppm may be considered as a significant acylation shift ΔδH(TAC).

It can be stated that the OTAC group produces analogous acylation effects as the OAc group, as regards the magnitude and stereospecificity. The ΔδH(TAC) values for paramagnetic α, β, γ shifts are about 0·1 ppm higher than the corresponding ΔδH(Ac). This difference must be taken into consideration mainly in the interpretation of the γ-shifts, in those cases when van der Waals effect, and hence, negative acylation shifts are expected. If the induced shift is not sufficiently large, it can be considerably affected by this paramagnetic contribution. Therefore the



A B

FIG. 5

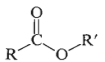
Comparison of Acylation Effects of the Acetyl Groups and the TAC Group, Obtained on Acylation of the $C_{(8)}$—OH Group of Substances with the Structural Formula A

The numbers in B at individual protons indicate the acylation shifts with R = acetyl and the numbers in brackets those with R = TAC.

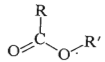
* In our communications[33–41] the opposite convention for signs was used, *i.e.* — for paramagnetic and + for diamagnetic shifts.

shifts $\geqq \pm 0{\cdot}1$ ppm must be considered as significant, especially in the case of stereochemical implications. As an example the comparison of the acylation effects of OAc and OTAC (in parentheses) groups in 3-oxo-8α-hydroxyeremophila-1,7-dien-8,12-olide is shown in Fig. 5. From the γ-shifts $\Delta^{(8)}\,\delta H_{6a}$ and $\Delta^{(8)}\,\delta H_{10a}$ it is evident how the 1,3-effect is distorted by the paramagnetic shift of the TAC-group. In such uncharacteristic cases it is necessary to check the TAC effects by comparison with the corresponding acetyl derivative (ref.[46]). A list of some useful TAI acylation effects is given in Table III.

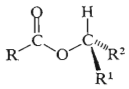
The similarity of the shielding effects of O-TAC and O-Ac groups indicates that both groups have the same conformation of the ester moiety and that no important additional effects of the NH—$COCCl_3$ group are involved. The conformations of the esters $R^1R^2R^3COCOR^4$ are shown in Fig. 6. On the basis of dipole moments the esters exist predominantly in Z-conformation[73]. This conformation was also found by X-ray analysis[74] of some acetates of secondary alcohols in the solid state. As



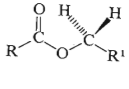
Z



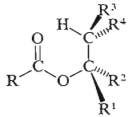
E



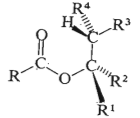
Z 1



Z 2



Z 3



Z 4

FIG. 6

Schematic Representation of *Z* and *E* Conformations of the Ester $R^1R^2R^3C—O—CO—R$

Z and *E* denote the basic conformations of the ester group, *Z1* and *Z2* denote the preferred conformations of esters of secondary and primary alcohols, *Z3* and *Z4* represent the conformation of a tertiary alcohol for which the largest β-shift can be expected.

shown by X-ray studies, among the possible C—O-bond rotamers the eclipsed rotamer[74] is preferred. From an ORD study[75] of steroid acetates if follows that such a conformational preference is evidently typical of acetates of secondary alcohols in solutions as well. Equally, the values of α-acylation ^{1}H-NMR effects of secondary alcohols are consistent with such conformational assumptions[76,77]. Assuming the acylation shifts are due to magnetic anisotropy of the ester group and mainly due to carbonyl group (in the first approximation) then the largest deshielding effect can be expected even for the eclipsed rotamer of the *Z*-conformation, *i.e.* in the conformation *Z1* in Fig. 6. On the other hand, in he case of primary alcohols the staggered rotamer *Z2* in Fig. 6 should be preferred for sterical reasons. Therefore smaller α-shifts result for primary alcohols[76]. These conformational assumptions are further consistent with the observed β-shifts. As it may be easily deduced from Dreiding models the best conditions for the observation of pronounced deshielding effects on protons in beta--positions exist in the *Z*-conformations in which the C(β)—H bond is synperiplanar or synclinal with the C(α)—O bond. This is schematically shown in conformations *Z3* and *Z4* in Fig. 6. The adaptation of such sterical arrangements is also probably the reason for the very large β-shifts of the order about 1 ppm observed in some tertiary alcohols[77]. The population of such conformations can also explain the large mean value of the β-acylation shifts of tertiary methyl groups protons of the type CH_3—C(R,R)—OH ($\Delta^{\beta}CH_3 = 0{\cdot}3-0{\cdot}5$ ppm). The only exception which we have observed so far, is the case when the tertiary methyl group and the tertiary hydroxyl group are bound in the α-position to the γ-lactone ring[39]. In such a case the absence of a distinct β-acylation shift of methyl signals was always observed, both for the acetate and the TAC derivative. This absence is probably a consequence of the destabilization of the *Z*-conformation in consequence of dipole–dipole interactions of the ester and the lactone group, and it may be assumed that in this case the *E*-configuration of the ester is preferred. The hypothesis that this is a specific feature of the γ-lactone ring is further confirmed by measurements of the acylation shifts of teriary methyl groups of the type CH_3—C—OH bound in the α-position of acyclic and cyclic ketones. In these cases we have observed the abovementioned "normal" β-shifts of 0·3 to 0·5 ppm. In conformations like *Z1* and *Z2* in Fig. 6 which are suitable for primary and secondary alcohols and for observations of pronounced alfa-shifts the conditions for observation of large beta-shifts mentioned above do not exist. This is in accordance

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[a] Average value; [b] ref.[10,12,29]; we observed the largest acylation shift 1·66 ppm for di-tert-butyl carbinol; [c] ref.[38,39,44–46]; [d] values for various fragments of the HO—C—CH_2— and HO—C—CH—type; values significantly larger then 0·5 ppm are typical only for tert.alcohols [e] ref.[9,37,38,45]; [f] ref.[11,12]; [g] depends on the relative configuration of H and OH; [h] the presented data are from a TAI-experiment with 2-propen-1-ol; [i] values for various fragments of the type CH_3—C(CH_3)—C—OH and CH_3—C—C—OH; [j] from TAI-experiment with 2-propyn-1-o [k] ref.[42,43]; [l] ref.[9]; [m] ref.[34,72].

TABLE III
Typical Values of TAI-Induced Acylation Shifts $\Delta^{(i)}\delta$H in Some Structural Types of Hydroxy Derivatives H—(C)$_n$—OH

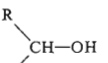
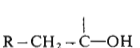
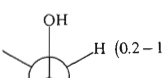
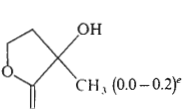
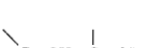
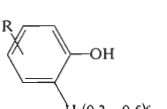
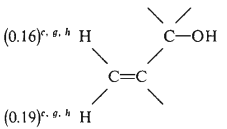
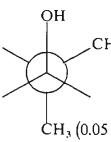
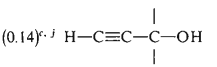
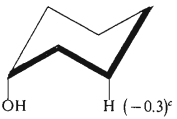
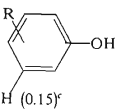
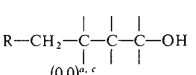
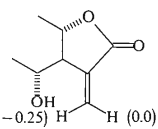
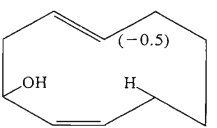
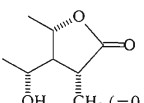
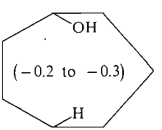
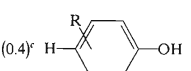
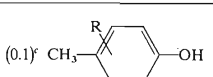
n (i)	$\Delta^{(i)}\delta$H
1 (α)	$R-CH_2-OH$ $(0.5-0.9)^{a,b}$  $(0.9-1.7)^{b}$
2 (β)	 $(0.15-0.2)^{a,c}$  $H\ (0.2-1.2)^{d}$ $H\ (0.0-0.2)^{d}$  $CH_3\ (0.3-0.5)^{e}$  $CH_3\ (0.0-0.2)^{e}$  $(-0.05)^{c,f,g,h}$  $H\ (0.3-0.5)^{e}$

TABLE III
(*Continued*)

n (i)	$\Delta^{(i)}\,\delta$H
3 (γ)	     
4 (δ)	  [HO ... H 0.25 nm]g, m  [HO ... H 0.2 nm]g, k  [HO ... CH3 0.25 – 0.3 nm]g, g  [HO ... H 0.25 nm]g, l 
5 (ε)	

with the magnitude of the observed beta-shift in primary and secondary alcohols which, are usualy of the order of 0–0·5 ppm. In a general case the absence of the conformational preference of the ester group should be always reckoned with (in ORD studies[75] this was always attributed to cases when the Cotton effect was not observed), and therefore it is necessary to consider the acylation effects as mean values, at least in the sense of the definition (*3*):

$$(\Delta^{(i)}\,\delta H_j)_{obs} = \langle \Delta^{(i)}\,\delta H_j \rangle = p_Z \cdot \langle \Delta_Z^{(i)}\,\delta H_j \rangle + p_E \cdot \langle \Delta^{(i)}\,jH_j \rangle\,, \qquad (3)$$

where p_Z and p_E are the fractional populations of the *Z* and *E* conformations of the ester group.

From Table II it follows that the values of the α, β and γ-acylation effects of the TAC group are not particularly dependent on the solvent, which is in agreement with the above mentioned assumption of the preference of the *Z*-conformation of the ester group, in consequence of the electron distribution of the ester group. Aromatic solvents are an exception in which the α- and the β-acylation effects are 0·10 to 0·20 ppm lower in consequence of the ring-current effect in selectively solvated ester groups. This effect is less pronounced in the α-acylation shift of the methine proton of the isopropyl group (in $CDCl_3$: $\Delta^z\delta H(TAC) = 1{\cdot}10$, in C_6D_6: $\Delta^z\delta H(TAC) = 1{\cdot}16$), as could be expected with respect to the above mentioned preferred conformation for secondary alcohols, in which the C_α—H and C=O bonds are synplanar (on the basis of the chemical shifts of 2-propanol and isopropyl acetate (in $CDCl_3$: Δ^z . . $\delta H(Ac) = 0{\cdot}98$, in C_6D_6: $\Delta^z\delta H(Ac) = 1{\cdot}28$ ppm). In the case of the OTAC group this paramagnetic shift is smaller than in the case of acetates, in consequence of the shift of the centre of the positive charge distribution, and thus also the solvation centre, farther from the C_α-atom, *i.e.* from the ester group to the CCl_3CONH— group.

So far, the conformation of the TAC group itself is not clear. According to our present experience in the presence of an excess of TAI the number of NH signals (if some co-reactions did not interfere) corresponded to the number of OH groups, which means, in the first approximation, that one of the possible conformations of the TAC group was always preferred. If indicating gradually in *Z* and *E* terms the conformations of the ester and the amide groups, then (under the assumption of the preference of the *Z*-conformation of the ester group) we can consider four conformers, *i.e. ZZZ* (*V*), *ZZE* (*VI*), *ZEZ* (*VII*) and *ZEE* (*VIII*). From ^{1}H-NMR studies[78] it is known that in diacylamines the conformations with quasiantiperiplanar arrangement of the C=O dipoles are preferred. Analogously for the TAC group the preference of the conformations *ZEE* and *ZZZ* can also be expected. From the viewpoint of steric effects the *ZZZ*-conformer seems more stable. If considering the probable polarisation of the molecule, as symbolized by the structure *Va*, then a certain preference may be assigned to this conformer even from the point of view of electrostatic interactions.

As is evident from Table II the acylation effects in ^{1}H-NMR spectra do not represent an unambiguous criterion for the structural assignment of hydroxy derivatives by themselves, as indicated in literature[29]. This is mainly true for the differentiation between secondary and tertiary OH groups. Here, the principial difficulty consists in the abnormally high β-shifts *cf.* tertiary alcohols, 0·9 – 1·2 ppm, comparable to α-shifts[77,79], observed in some configurational arrangements (axial-equatorial and *vice versa*). Thus the acylation of a teriary hydroxyl can simulate in some cases an α-shift of a secondary OH. A similar ambiguity is also met in the case of phenolic derivatives when solving the problem of the position of OH on the benzene nucleus, due to the similarity of the *ortho*- and *para*-acylation shifts (ref.[80,81]). Large β-shifts should be carefully considered especially if TAI-experiments are applied on polyfunctional derivatives. Recent studies have shown[29,82] that the problem of the assignment of acylation effects of secondary and tertiary OH is well solvable by the TAI--method in combination with ^{13}C-NMR, where the ^{13}C shifts of the C_α of tertiary alcohols are distinctly different from other cases (disregarding a few exceptions).

The TAI acylation effects can be used for rapid solutions of various structural problems, as demonstrated by experiments in Figs 2 – 5, 7 – 9 and in Table III. When interpreting the TAI acylation effects, as well as the acylation effects in general, it should always be kept in mind that their value and stereospecificity are dependent on conformational factors. Therefore it is necessary to judge their characteristics always using the whole set of the acylation shifts induced, so that the character of the shielding field of the acyl group could be estimated. In addition to the already mentioned problem of the conformational preference of the ester group (this preference can be generally different *e.g.* in poly-TAC-derivatives the conformations of the TAC groups can affect each other) an important condition for the characteristicity of the acylation shifts is the requirement that the molecular conformations of the hydroxy derivative and of its ester – *i.e.* the conformation of the affected part of the molecule – should be identical. In view of the fact that the OH group is bulky due to the "effectively" large oxygen atom, it often happens in conformationally mobile systems that the alcohol exhibits a dynamic conformation, while a fixed conformation is reached only after acylation when the van der Waals effect is eliminated by the partial delocalization of electron lone-pairs in the ester group (*cf.* for example ref.[35]). For the same reason the dependence of the β-shielding effect of such a multipole (as the acyl group is) on the dihedral angle, such as it was discussed in some cases[83], can be determined with difficulty. Therefore the additivity

$$\Delta^{(i,j,k,\ldots)}\,\delta H_r = \Delta^{(i)}\,\delta H_r + \Delta^{(j)}\,\delta H_r + \Delta^{(k)}\,\delta H_r + \ldots \qquad (4)$$

does not apply generally.

The pronounced paramagnetic β-shifts and diamagnetic γ, δ or other transanular van der Waals effects are of special utility. As an example of β-shifts TAI acylation of

laserpitin, a sesquiterpenic substance of carotane type, is shown in Fig. 7. The acylation of a tertiary hydroxyl group in the position α to the carbonyl group induces distinctly different paramagnetic β-shifts of neighbouring geminal protons than may be expected from the typical steric arrangement shown in Table III. Simultaneously a significant paramagnetic β-shift of the signal of the tertiary methyl group is also observed. The acylation of the second tertiary hydroxyl induces a large paramagnetic β-shift of the methine proton signal of the isopropyl group, under simultaneous decrease of the 2nd order effect in both doublets of the secondary methyl groups. Such changes of the signals of the isopropyl group, as well as the mentioned β-shifts of tertiary methyls of the CH_3—C—OH type, can be utilized with advantage for the assignment of the signals of tertiary and secondary methyl groups, especially in those cases when a larger number of such signals is present in the spectrum.

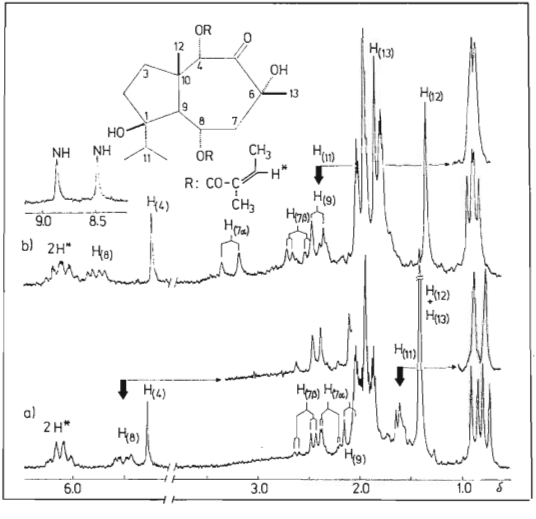


FIG. 7

Application of TAI-Induced β-Acylation Shifts for Structural Assignment of Methyl Groups

The bottom trace a) shows the starting spectrum and two key frequency-swept decoupling experiments (the irradiated position is indicated with a thick arrow), while the upper trace b) is the spectrum after addition of an excess of TAI.

As a further example the above-mentioned problem of the assignment of the signals of two geminal dimethyl groups of the $(CH_3)_2$—C—OH type can be mentioned which can also be solved directly on the basis of the values of the acylation β-effects. It is possible to assume that both signals belonging always to one geminal dimethyl group will have approximately the same paramagnetic β-shift, and they can thus be assigned directly on the basis of the comparison of the starting and the final spectrum (Fig. 3). In the given case comparable acylation shifts occur of the order 0·4 ppm and 0·3 ppm for the pairs of methyl signals, in the assignment shown in Fig. 2. As mentioned before, the characteristic β-shift for the signals of the tertiary methyl of the type CH_3—C—OH is not present in the case when the C atom is also simultaneously in the α-position to the lactone carbonyl of the γ-lactone ring, probably because the ester group is not in the preferred *Z*-conformation. This fact can be used with advantage for the solution of the position of the tertiary hydroxy groups in sesquiterpenic lactones, especially in guaianolides[38,45]. As indicated by LIS experiments with these compounds the tertiary methyl groups of the CH_3—C—OR type have comparable relative gradients at R = H and R = $COCH_3$ and the solution of the problem of the assignment of the tertiary methyl groups and tertiary OH groups by means of LSR is less straightforward than in TAI experiments[45].

In the case of the acylation of H—$(C)_n$—OH, for $n \geqq 3$, the diamagnetic acylation shifts observed so far, *cf.* Table III, could be interpreted on the basis of the van der Waals effect[84,85]. For the OH group the value of this effect can be estimated as 0·6 to 0·2 ppm for the distances 0·2 – 0·25 nm (ref.[72]). On acylation of OH the lone pairs

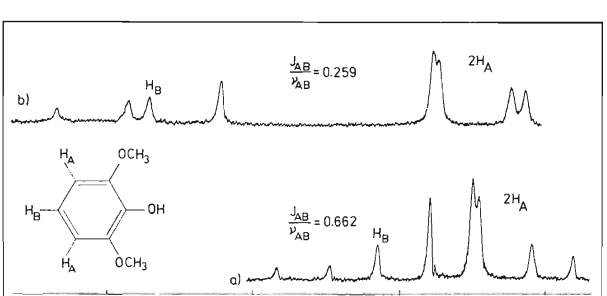


FIG. 8

TAI Acylation of 2,6-Dimethoxyphenol (measured at 60 MHz; solvent deuteriochloroform) a) Starting spectrum, b) after acylation with TAI.

of the ethereal O-atom of the ester group are delocalized, which results in a decrease of polarizability and the ionizing potential of this O atom as the perturbing group and in subsequent elimination of the van der Waals component of the shielding field. In view of the isotropic character of this effect the observation of significant diamagnetic (upfield) shifts is always very useful, especially for stereochemical implications.

In phenols the effect of the TAC group is transferred relatively far, so that significant acylation shifts can be observed even on protons up to 5 bonds from the C atom. As a simple example of the acylation of phenol with TAI its effect on the spectrum of 2,6-dimethoxyphenol is shown in Fig. 8. This example illustrates clearly the function of TAI as a shift-reagent.

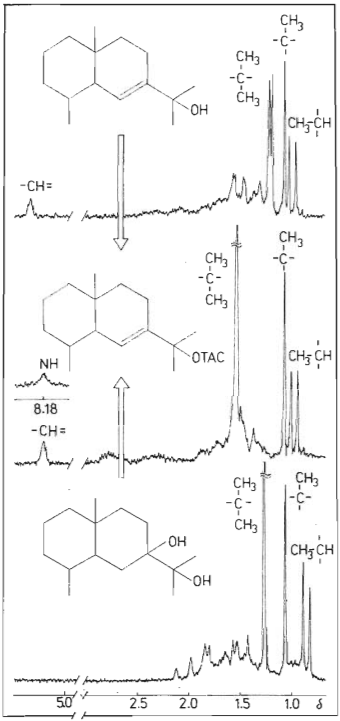


FIG. 9

In situ Elimination Reaction Induced with TAI and Its Use for Correlation of Two Sesquiterpenic Alcohols

The experiments with hydroxy compounds containing an OH group bound by a strong intramolecular hydrogen bond of the type—NO_2...H—O— or C=O...H—O—, as for example in *o*-nitrophenol, 7-methoxyeuparin[41] and acetyl acetone, have shown that such hydroxy groups are very little reactive with respect to TAI. With other types of intramolecular bonds (for example O—H...O—H) we could not observe this lack of reactivity. It is evidently the consequence of the resonance stabilization of the trigonal configuration of the O atom of the OH group (which is unfavourable for an attack by TAI).

The elimination reactions of the OH groups during the *in situ* acylations with TAI, observed in some cases, are interesting. So far only rare cases are known and it is not evident which are the additional conditions for their systematic occurrence during TAI experiments. Anyway, these reactions can be used with advantage in suitable cases for structural correlations *in situ*. The types of elimination reactions observed so far are given in Table I (entries *36–38*). As an example an in situ correlation of two natural selinane derivatives are shown in Fig. 9.

Elimination reactions, as well as the above discussed partial or stepwise acylations, are also a clear demonstration of potential use of the *in situ* reactions for the modelling of preparative reactions. Thus, the *in situ* reactions in the NMR sample-tubes obtain a chemical dimension.

EXPERIMENTAL

Standard Procedure for Monohydroxy Compounds

The ^{1}H-NMR spectrum of substrate is measured in a suitable solvent and the analysis of the spectrum is carried out. A few drops of TAI (excess) are then added to the solution in the sample tube at room temperature and the spectrum is measured and analysed again. The amount of substrate should be as small as possible,so that the requirements for the amount of TAI also could be minimum and the solvent would be able to moderate the heat of reaction. In the case of the CW-technique on a 60—100 MHz instrument about 10—20 mg of substrate suffice (depending on the size of the molecule), dissolved in about 0·3—0·4 ml of solvent. For the signals of tertiary methyl groups of the CH_3—C—OH type about 1 mg sample suffices for the observation of their acylation shifts. The time necessary for the experiment depends on the reactivity. With reactive hydroxyls the reactions are mostly terminated before the first spectrum is recorded. When observing a partial acylation and when the spectrum does not further change in time, further TAI is added. In the case of slow reaction the mixture is allowed to stand at room temperature and under exclusion of air humidity until the reaction is over. If no detectable change of the spectrum can be observed after one week of standing the result is negative. In the case of a strongly exothermic reaction tetramethylsilane often escapes from the solution. If a co-reaction under elimination of CO_2 is possible it is suitable to repeat the reaction in the presence of a higher boiling standard (hexamethyldisiloxane). In some solvents, especially when concentrated substrate solutions are used, a precipitate can be formed on addition of TAI, due to a lower solubility of the reaction products. The precipitate can be dissolved by addition of more solvent or by using an other solvent. The solvents usually containing higher amounts of water, as for example dimethyl sulfoxide or acetone, should be dried over a molecular sieve.

Titration Experiment with Polyhydroxy Compounds or with Mixtures of Hydroxy Compounds

These differ from the preceding standard experiment by a gradual addition of TAI in very small doses, especially at the beginning of the experiment. In our practice, the addition of TAI from a glass bubble-pipette with a capillary tip was found convenient, because the tip can be easily sealed after each addition. The process of addition is monitored by the observed course of the reaction.

The experiments were carried out on VARIAN HA-100 (100 MHz) and TESLA BS 467 (60 MHz) instruments. Trichloroacetyl isocyanate (Merck) was employed. The investigated substances of natural origin are defined in respective references. The solvents were dried over molecular sieve Nalsit 4.

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