DIETHYLAMINE ADDITION TO NATURAL SESQUITERPENIC α-EXOMETHYLENE-γ-LACTONES AND ITS USE FOR CHEMICAL TRANSFORMATIONS OF THESE COMPOUNDS*

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Addition of diethylamine to exomethylene double bond in natural sesquiterpenic α-methylene--γ-lactones is more advantageous in structural studies than the addition of other amines used so far. In some instances a simultaneous solvolysis of the ester groups also takes place. This fact was made use of for selective deacylation. The possibility of solvolysis impairs the potential use of diethylamine and other amines for the isolations of α-methylene-γ-lactone fractions directly from the extract of natural material.

The formation of the adducts of ammonia or dimethylamine with lactones containing α-exomethylene double bond was used for the isolations and the characterizations of these substances[1]. In connection with the discovery of the biological activity of sesquiterpenic α-methylene-γ-lactones, interest in the preparation of structural analogues of these substances increased, carried out by chemical transformation or total synthesis. Therefore the possibility of the formation of adducts was also investigated, either with hydroxyls (irreversible addition of methanol was the cause of the formation of many artifacts during the isolations of sequiterpenic α-methylene--γ-lactones or the saponifications of their esters), or with amines[2] or thiols[3]. The reversible Michael addition of amines and thiols was made use of for the protection of the exomethylene double bond, most often with 1-propanethiol[3] and dimethylamine[4,5], and in some cases with morpholine[6] as well.

In connection with the structural analyses of sesquiterpenic lactones we studied the utilization of chemical transformations aiming at their use in ^{1}H NMR spectroscopy[7]. We prepared substances suitable for the checking of the validity of the lactone rule[8], using transformations of skeletal groups of natural sesquiterpenic lactones with simultaneous preservation of the exomethylene double bond. We specially investigated reactions suitable for the protection and the regeneration of the exomethylene group. From the practical point of view it seemed purposeful to use

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diethylamine, since in addition to an easy formation of the adducts it also permitted the splitting off of the protecting group by means of silica gel[4]. We used the addition of diethylamine for the protection of the exomethylene group in transformations of natural compounds in an earlier study[7]. In this paper we shall discuss in detail the practical aspects of the addition and its utilization.

Protection with dimethylamine or morpholine was suitable for the work with larger quantities of substances when crystallization could be used for the separation and the purification of the products, since a chromatographic separation of the adducts was accompanied by difficulties ensuing from their relatively high instability in contact with silica gel. However, when working with small amounts, the use of diethylamine has several adavantages over that with dimethylamine or morpholine. Its properties guarantee an easy work-up of the reaction mixture and its chromatographic separation. The use of diethylamine in the chromatographic solvent system during the separation of the diethylamine adducts substantially limits the splitting off of the protecting group even during chromatography and improves the separation on alumina.

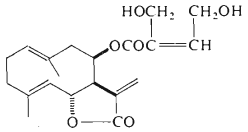
The regeneration of the exomethylene double bond is currently carried out by quaternization of the amine adduct with methyl iodide and subsequent treatment with sodium hydrogen carbonate. This method, originally used in one of the first syntheses of α-methylene-γ-lactones[9], was later used in one of the protecting groups, both amine groups[4] and thiol groups[3]. In experiments with chromatographic purification of the products of addition of diethylamine to eupatoriopicrin (*I*) we already observed an elimination of the diethylamine group on the silica gel column during chromatography with chloroform. The course of the elimination was investigated in detail with the diethylamine adduct of eupatolide (*II*) and it was used for the regeneration of the exomethylene double bond even in other cases[7]. The splitting off of the protecting group was carried out in chloroform on silica gel containing 12% of water.

A similar procedure was also used in the conversion of costunolide[4]. For the protection of the exomethylene group dimethylamine was used, followed by thermolysis. The product consisted of a mixture of substances with an already thermolytically regenerated exomethylene group. The unreacted part of the dimethylamine adduct of costunolide was converted to costunolide by means of silica gel in chloroform. In another type of amine adducts of some further sesquiterpenic α-methylene-γ--lactones the splitting off of the protecting group during chromatography was not observed. For example, a mixture of the adducts of morpholine with the isomers of isoalantolactone was separated chromatographically on silica gel in light petroleum–ethyl acetate, without any observable elimination of morpholine[6]. Similarly a mixture of the adducts of morpholine with two melampolides, obtained by oxidation of costunolide with selenium dioxide[10], was also separated chromatographically. A series of various amine adducts was prepared with alantolactone and gross-

heimin[11]. Their purification was also carried out chromatographically, without elimination[11,12]. Probably in all instances a silica gel was used which was adjusted for column chromatography of neutral oxygen-containing substances of considerable polarity, deactivated with a certain amount of water. The regeneration of the exomethylene double bond on silica gel is evidently dependent on the type of the amine used, on the type of lactone and the choice of solvents. So far the elimination was observed with adducts of dimethylamine and diethylamine with lactones of the germacranolide (costunolide, eupatolide) and heliangolide type (3-dehydronobilin, eucannabinolide), and that only when chloroform was used as solvent.

In the preparation of diethylamine adduct from eupatoriopicrin (*I*) a quantitative solvolysis of the ester group close to the lactone ring was observed, under formation of the diethylamine adduct of eupatolide (*III*) as the main product, and also a small amount of methoxy derivative *IV*. This fact complicates the use of diethylamine for the protection of exomethylene on one hand, but on the other it represents a possibility of a selective elimination of the ester groups, so far carrierd out by base-catalysis[13]. The hydrolytic cleavage is usually non-selective in polyacyl derivatives, but it is usually accompanied by a number of side-reactions, for example relactonization with another, hydrolytically liberated hydroxyl group, addition of water or alcohol to the exomethylene group, and transannular cyclization. Therefore we checked the reaction of diethylamine with other sesquiterepnic lactones of the germacranolide (*I*–*VI*) and heliangolide type (*VII*–*XI*) with a lactone ring closed at $C_{(6)}$ and with various ester groups in the neighbourhood of the lactone ring in both possible configurations, α and β. Alatolide (*V*) afforded adduct *VI*, while 3-dehydronobilin (*VII*) afforded adduct *VIII*, without a solvolysis of the ester group. This indicates that the observed solvolysis is evidently specific of the acid residue and the β-configuration of the ester at $C_{(8)}$. This conclusion is supported by the result of the addition to eucannabinolide (*IX*) which contains the same β-oriented α,β-*cis*-bis(hydroxymethyl)-acryloyloxy group in the position $C_{(8)}$ as eupatoriopicrin (*I*). After the splitting off of the diethylamino group on silica gel we obtained predominantly 8-deacyleucannnabinolide (*X*), confirming the assumed solvolysis. The solvolysis with diethylamine represents a further possinility of the elimination of the ester side chain for this case, because 8-deacyleucannabinolide (*X*) has been obtained so far by base-catalyzed hydrolysis only in a single case[14]. In all other cases[15–17] only the methoxy derivative *XI* was obtained.

On the other hand, solvolysis should be also considered when the amino adducts are used for the isolation of α-methylene-γ-lactones from mixtures or from natural sources (for example by extraction. crystallization, chromatography or selective binding and removal on polymeric nucleophiles of amine character[18]), and then checked whether the substance obtained in this manner is natural product or an artifact.



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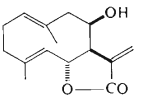

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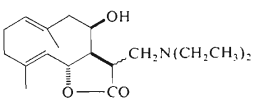

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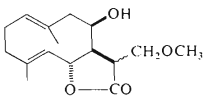

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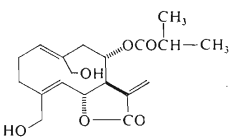

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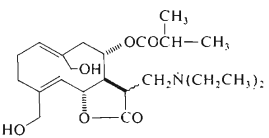
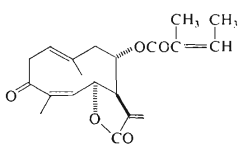


VI



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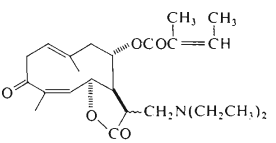

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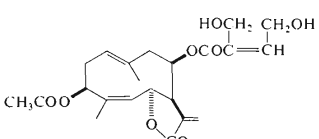
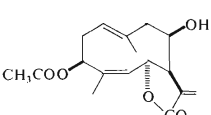


IX



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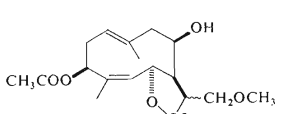

XI

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The molecular masses were determined by mass spectrometry on an AEI MS 902 instrument. The ^{1}H NMR spectra were recorded with a Varian HA 100 and Varian XL-200 spectrometer in chloroform, using tetramethylsilane as internal reference. For thin-layer chromatography Kieselgel GF_{254} (Merck) and Alumina G (Woelm) were used. For column chromatography and for the elimination of the protecting groups Kieselgel (Herrmann, Köln) and silica gel according to Pitra (service laboratories of this institute in Lysolaje), fractionated by sedimentation, dried and deactivated with 12% of water were used. Eupatoriopicrin (*I*) and eucannabinolide (*IX*) were isolated from *Eupatorium cannabinum*[19].

Addition of Diethylamine to α-Exomethylene-γ-lactone

A freshly distilled diethylamine (10 ml) was added in portions to a solution of the compound investigated (200 mg) in methanol (5 ml), cooled at 0°C. The solution was allowed to stand in an ice bath as long as heat was evolved (about 10 min) and then at room temperature overnight. When less than 20 mg of substance were used the solution was not cooled either before or during the reaction. The course of the addition was monitored by thin layer chromatography in chloroform–ethyl acetate–methanol or cyclohexane–ether mixtures, in ratios corresponding to the polarity of the substance investigated. After the termination of the addition, quantitative in most instances, the mixture was evaporated under reduced pressure and the residue was crystallized from a small amount of methanol. In cases when the adduct would not crystallize they were purified or separated chromatographically on a short column of alumina (act. III) using benzene–diethylamine (50 : 1 to 20 : 1) for elution. When such a purification proved unsatisfactory, it was carried out on a short column of silica gel, using cyclohexane–diethyl ether–diethylamine (6 : 4 : 1) for elution.

Regeneration of α-Exomethylene-γ-lactones from the Adducts by Diethylamino Group Elimination on Silica Gel with Chloroform

Silica gel (5 g) was added to a solution of the adduct (100 mg) in chloroform (10 ml) and the suspension was stirred overnight. The elimination of the diethylamino group was monitored by thin-layer chromatography. The suspension was transferred into a small chromatographic tube and the product was eluted with chloroform–ethyl acetate (10 : 1). After evaporation the residue was further separated chromatographically.

Addition of Dimethylamine to Eupatoriopicrin (*I*)

The product (300 mg) was crystallized from methanol. The adduct *III* obtained in this manner (200 mg) had m.p. 145–147°C, M^+: 321 (mass spectrometry); ^{1}H NMR spectrum: 5·13 t ($J_{6,5} = 9{\cdot}8$, $J_{6,7} = 9{\cdot}4$, $H_{(6)}$); 4·79 bdd ($J_{1,2a} \approx 11$, $J_{1,2b} \approx 4{\cdot}5$, $H_{(1)}$); 4·65 bd ($J_{5,6} \approx 9{\cdot}8$, $H_{(5)}$); 4·56 bd ($J_{8,9} \approx 4{\cdot}5$, $H_{(8)}$); 1·70 bs ($H_{(15)}$); 1·61 bs ($H_{(14)}$); 2·52 m ($N{-}CH_2$); 1·00 t ($J = 7{\cdot}1$, $N{-}CH_2CH_3$). For $C_{19}H_{31}NO_3$ (321·4) calculated: 70·99% C, 9·72% H, 4·36% N; found: 70·10% C, 9·76% H, 4·68% N.

On elimination of the diethylamino group eupatolide (*II*) was obtained, identical with authentic eupatolide according to thin-layer chromatography, mass spectrum and ^{1}H NMR spectrum. The mother liquors after crystallization of adduct *III* (after elimination) were separated chromatographically on a silica gel column with benzene–ethyl acetate (20 : 1 to 5 : 1). Further, eupatolide

(*II*) was obtained, as well as a small amount (5 mg) of its methoxy derivative *IV*, known earlier[20,21], with m.p. 138–139°C, identified by means of mass spectrum, M^+: 280, with elemental composition $C_{16}H_{24}O_4$, further *m/e*: 262 ($M-H_2O$), 248 ($M-CH_3OH$), 235 ($M-C_2H_5O$), 230 ($M-H_2O-CH_3OH$), 217 ($M-H_2O-C_2H_5O$). ^{1}H NMR spectrum: 5·16 t ($J_{6,5}$ = 10, $J_{6,7} \approx 9{\cdot}5$, $H_{(6)}$); 4·81 um ($H_{(1)}$); 4·68 bd ($J_{5,6}$ = 10, $H_{(5)}$); 4·36 um ($H_{(8)}$); 3·61 dd and 3·73 dd ($J_{13,13}$ = 10, $J_{13,11} = 3{\cdot}5 + 3{\cdot}5$, $H_{(13)}$); 3·34 s (OCH_3); 2·93 dt ($J_{11,7}$ = 12, $J_{11,13} = 3{\cdot}5 + 3{\cdot}5$, $H_{(11)}$); 1·69 d ($J \approx 1{\cdot}5$, $H_{(15)}$); 1·61 bs ($H_{(14)}$).

Addition of Diethylamine to Eupatolide (*II*)

The product (20 mg) was identical with the adduct *III* obtained from eupatoriopicrin (*I*), according to its m.p., mass and ^{1}H NMR spectra.

Addition of Diethylamine to Alatolide (*V*)

The product (70 mg) was purified on a short silica gel column using benzene–acetone–diethylamine mixture (20 : 1 : 1) and then evaporated. It was identified as adduct *VI*. ^{1}H NMR spectrum: 5·24 td ($J_{8,9a}$ = 8·8, $J_{8,9b}$ = 8·5, $J_{8,7}$ = 2·8, $H_{(8)}$); 5·08 dd ($J_{6,5}$ = 9·9, $J_{6,7}$ = 8·9, $H_{(6)}$); 5·15 dd ($J_{1,2a}$ = 11·6, $J_{1,2b}$ = 4·4, $H_{(1)}$); 4·86 d ($J_{5,6}$ = 9·9, $H_{(5)}$); 4·31 d and 3·98 d ($J_{15,15}$ = 13·9, $H_{(15)}$); 4·26 d and 3·91 d ($J_{14,14}$ = 12·2, $H_{(14)}$); 3·15 dd and 2·85 dd ($J_{13,13}$ = 13·8, $J_{13a,11}$ = 1·7, $J_{13b,11}$ = 5·5, $H_{(13)}$); 2·97 dt ($J_{7,11}$ = 11·5, $J_{7,6}$ = 8·9, $J_{7,8}$ = 8·3, $H_{(7)}$); 2·56 m (N—CH_2); 1·02 t (J = 7·15, N—CH_2—CH_3); 1·19 d and 1·18 d (J = 7·0, 2 × CH_3). For $C_{23}H_{37}NO_6$ (423·5) calculated: 65·22% C, 8·81% H, 3·31% N; found: 64·73% C, 8·85% H, 3·76% N. Alatolide (*V*), identified by thin-layer chromatography and ^{1}H NMR spectroscopy, was obtained on elimination of the diethylamino group.

Addition of Diethylamine to 3-Dehydronobilin (*VII*)

The product (50 mg) was purified on a short column of alumina with a mixture of benzene and diethylamine (50 : 1) and evaporated. The adduct *VIII* obtained in this manner had M^+: 417 (mass spectrometry). ^{1}H NMR spectrum: 5·48 bd (J = 10, $H_{(5)}$); 5·45 m ($H_{(1)}$); 5·02 m ($H_{(8)}$); 4·17 bd ($J_{5,6}$ = 10, $H_{(6)}$); 3·16 d (J = 8·5, $H_{(2)}$); 1·89 ($H_{(15)}$); 1·79 ($H_{(14)}$); 1·97 dq (α-CH_3); 1·88 qq (βCH_3); 6·13 qq (βH); 2·70 m (N—CH_2); 1·02 t (J = 7, N—CH_2CH_3). For $C_{23}H_{35}NO_5$ (417·5) calculated: 69·03% C, 8·45% H, 3·35% N; found: 68·85% C, 8·33% H, 3·55% N. Elimination of the diethylamino group led to 3-dehydronobilin (*VII*), identified by thin-layer chromatography and ^{1}H NMR spectroscopy.

Addition of Diethylamine to Eucannabinolide (*IX*)

The product of the addition (30 mg) was not isolated but directly submitted to diethylamino group elimination. The product of elimination was chromatographically separated on silica gel with chloroform–ethyl acetate–methanol (100 : 5 : 1). From the main fraction 8-deacyleucannabinolide (*X*) was obtained, M^+: 306 (by mass spectrometry); ^{1}H NMR spectrum: 6·37 d ($J_{13a,7}$ = 2·5, $H_{(13a)}$); 5·68 d ($J_{13b,7}$ = 2·1, $H_{(13b)}$); 5·83 dd ($J_{6,5}$ = 10·8, $J_{6,7}$ = 2·6, $H_{(6)}$); 5·23 m ($H_{(3)}$); 5·18 dq ($H_{(5)}$); 5·13 m ($H_{(1)}$); 4·19 bt (J = 3·2, $H_{(8)}$); 2·84 m ($H_{(7)}$); 2·56 dd ($J_{9a,9b} \approx 15$, $J_{9a,8}$ = 3·5, $H_{(9a)}$); 2·35 dd ($J_{9b,9a} \approx 15$, $J_{9b,8}$ = 3·5, $H_{(9b)}$); 1·90 bs ($H_{(14)}$); 1·81 d ($J_{15,5}$ = 1·5, $H_{(15)}$).

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