PHYTOECDYSTEROIDS OF *Silene nutans*.

II. 22-DEOXYECDYSTERONE AND FEATURES OF ITS MASS SPECTRUM

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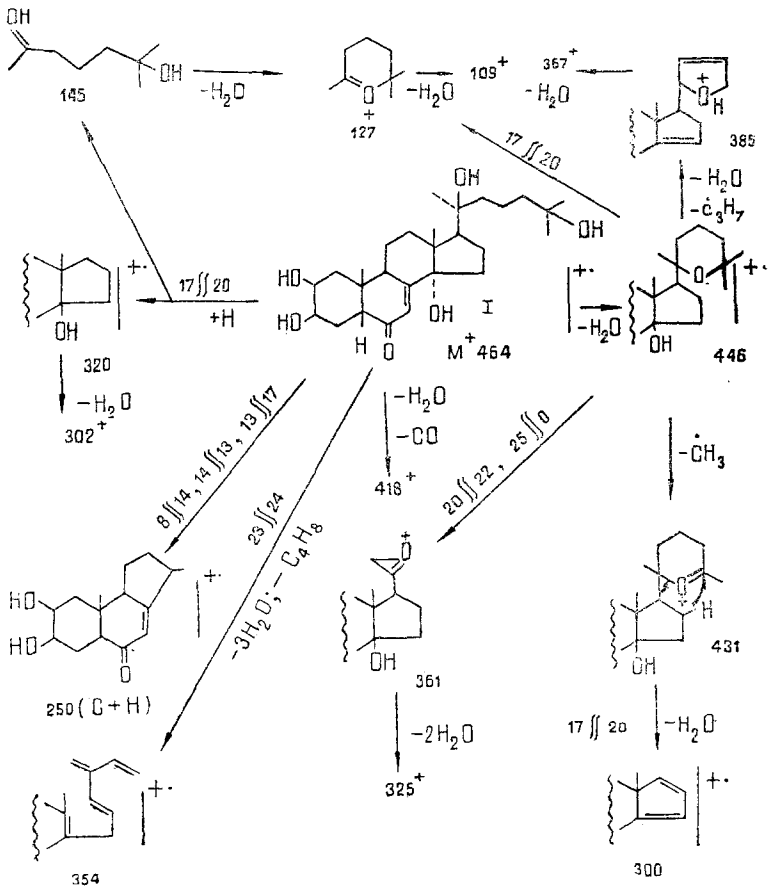
Using high-performance liquid chromatography, 22-deoxyecdysterone has been isolated from the butanolic fraction of the extractive substances from the epigeal part of *Silene nutans* L. Characteristic features of the mass spectrum of 22-deoxyecdysterone due to the absence of an oxygen function at C-22 are discussed.

We have previously [1] reported the use of high-performance liquid chromatography (HPLC) for the separation of the phytoecdysteroids from *Silene nutans* L. (syn. *Melandrium nutans*). Ecdysterone and polypodin B were isolated in the individual state. A more complete utilization of the possibilities of HPLC has permitted the isolation from this plant of another compound, with the composition $C_{27}H_{44}O_6$ (I). Its assignment to the ecdysteroids was confirmed by its UV and IR spectra: $\lambda_{max}^{C_2H_5OH}$ 244 nm (log ε 4.03); 3400-3430 cm^{-1} (OH), 1647 cm^{-1} (conjugated ketone). The capacity factor of the substance on HPLC for a reversed-phase column was 2.72 (its maximum appeared after that of ecdysterone).



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The mass spectrum of compound (I) contained the peak of the molecular ion with m/z 464. The presence of the head ion of series *a* with m/z 363 and the products of its dehydration with m/z 345, 327, and 309, and also of the ions of series b [2, 3] with m/z 301, 300, and 299 indicated the presence in the ecdysteroid under investigation of a tertiary OH group at C-20 and of three OH groups in the steroid nucleus. Ions with m/z 250 and 249 (series c) confirmed that two of the three hydroxyls were present in rings A-C.

In the PMR spectrum of the ecdysteroid (I) the signals of three protons appeared in the 3.0-4.1 ppm region: At 3.41 ppm the signal of a proton located at C-9 appeared clearly, and at 4.04 ppm a broadened multiplet of two protons at C-2 and C-3. The signal of the C-21 methyl group appeared at 1.41 ppm in the form of a singlet, and not of a doublet. We were therefore justified in assuming that one of the hydroxy groups was present at C-20.

In the mass spectrum of the compound that we had isolated, the peak of an ion with m/z 99, which is extremely characteristic for compounds close in the structure of the side chain to ecdysterone, was absent. This showed that there was no hydroxy group at C-22 or C-25. A double signal at 1.22 ppm of the two methyl groups at C-26 and C-27 was characteristic for ecdysteroid-like compounds with an OH group at C-25. And in the 3.60-3.90 ppm region, where a proton geminal to hydroxyl at C-22 resonates in the majority of natural ecdysteroids, there was no signal. This indicated the absence of a hydroxy group at C-22 in compound (I).

Attention is attracted by the presence in the mass spectrum of the ecdysteroid (I) of a series of intense peaks with m/z 145, 127, and 109 (100%). The mass numbers of the ions mentioned permit the assumption that they characterize the side chain at C-17, and each successive ion in this series is, judging from the presence of metastable peaks, a product of the dehydration of the preceding one. On the basis of the fact that compound (I) belongs to the series of C-27 ecdysteroids, the side chain must contain eight carbon atoms. In fact, in the high-resolution mass spectrum, the ion with m/z 109 was 100% composed of C_8H_{13} particles, and the ion with m/z 127 was a triplet 75% of the total height of which corresponded to $C_8H_{15}O$ ions. And although in the triplet of ions with m/z 145 there was only ~20% of the ion with the required composition, $C_8H_{17}O_2$, its affinity with the ions having m/z 127 and 109 is evidence in favor of the assumption that it was formed as the result of the simple cleavage of the C-17—C-20 bond (scheme).

The circular dichroism curve of ecdysterone (I) was characterized by negative and positive Cotton effects with $\Delta\varepsilon = -3.87$ ($\pi\rightarrow\pi^*$ transition, 246 nm) and $\Delta\varepsilon = +1.26$ ($n\rightarrow\pi^*$ transition, 338 nm), which shows the presence of a 5β(H)-Δ^7-6-keto-14-hydroxy grouping with the cis linkage of rings A/B.

The combination of all the facts mentioned permitted us to consider that the compound that we had isolated was 22-deoxyecdysterone.

The absence of an oxygen function at C-22 led to the appearance of a whole series of special features in the mass spectrum of the substance described. In the first place, numerous forms of cleavage of the C-17—C-20 bond appeared. In addition to the fact that one of them led to an ion with m/z 145 and the products of its fragmentation, the formation of ions of the steroid skeleton accompanied by the migration of hydrogen from the side chain was also observed. Such a process has been reported previously only in certain ecdysteroid acetates [3]. The ancestor of this series was an ion with m/z 320 ($C_{19}H_{28}O_4$).

The spectrum of the product of the deuterium exchange of ecdysteroid (I) with CD_3OD showed the practical absence of an isotopic shift for the ion with m/z 127. This means that this ion has a cyclic form regardless of whether it was formed by dehydration with the cyclization of the m/z 145 ion or appeared as the result of cleavage of the C-17—C-20 bond in the $(M - H_2O)^+$ ion with m/z 446 in which the side chain had previously been cyclized (see scheme). We have observed a similar process previously in the spectrum of integristerone B [4]. However, in the compound given as an example, because of the presence of an OH group at C-22 and the alternative cyclization with the participation of the hydroxyls at C-22 and C-25, the contribution of the ions with m/z 143 and 125 (analogous to the ions with m/z 127 and 109 for (I)) was far less considerable.

The idea of the cyclization of the side chain proved to be useful also for explaining some other features of the spectrum of 22-deoxyecdysterone. We may note, in particular, the presence in the spectrum of a series of ions containing C_{21} but having two atoms of hydrogen less than the ions of series *a* (m/z 361, 343, 325). The deficiency of hydrogens is

possibly a consequence of the fact that a cyclic form of the $(M - H_2O)$ fragment acted as the precursor of these ions (see scheme). An analysis of the most stable ion of this series, with m/z 325, by the method of defocusing the ion beam showed that in addition to $(M - nH_2O)^+$ fragments, other breakdown products — in particular, an ion with m/z 367 ($C_{24}H_{31}O_3$) — acted as its precursors. This ion, as shown in the scheme and confirmed by defocusing, was formed by the elimination of a C_3H_7 particle from the cyclized forms of the $(M - nH_2O)^+$ ions.

Also characteristic for the spectrum of compound (I) was the absence of $(M - C_5H_{11}O)^+$ ions corresponding to the cleavage of the C-22—C-23 bond, which have been detected in the spectra of a number of ecdysteroids with a OH group at C-22 [3]. At the same time, as a consequence of this cleavage here there was the strong peak of a $C_5H_9^+$ fragment with m/z 69. So far as concerns the breakdown of the side chain at the C-23—C-24 bond, it took place in 22-deoxyecdysterone by approximately the same route as in 20-deoxy and 20,22-dihydroxysterones [3] and led to an odd-electron fragment with m/z 354.

Experiments on the defocusing of the key fragments of the spectrum of 22-deoxyecdysterone of series *a*, b, and c, as previously in experiments with other phytoecdysteroids [5], confirmed the stepwise nature of the formation of these ions. It was found here, for example, that an ion of the series with m/z 300 in the spectrum of 22-deoxyecdysterone, like the analogous ion with m/z 284 in the case of 2-deoxy-α-ecdysone, had as its main precursor the fragment $(M - H_2O - CH_3)^+$. The mechanism of this process can be represented in the same way as in the scheme for the m/z 300 ion but, again starting from the cyclized form of the $(M - 33)^+$, m/z 431, ion.

22-Deoxyecdysterone has also been found in the leaves and stems of *Taxus cuspidata* (family Taxaceae). Nakano et al. [6] acetylated one of the fractions of a methanolic extract of the plant and, by saponifying the acetate, obtained in amorphous form a product which, according to spectral characteristics, had the structure of 22-deoxyecdysterone. The native nature of the new ecdysterone was established by a comparison in thin-layer chromatography of samples obtained directly from the methanolic extract and by the saponification of the acetate.

EXPERIMENTAL

General Remarks. IR spectra were obtained on a UR-20 spectrophotometer (KBr), and PMR spectra on a JNM-4H-100 instrument at a sample temperature of 22 ± 2°C, the results being given on the δ scale with 0 — HMDS. For chromatography we used L silica gel (Chemapol, Czechoslovakia). Mass spectra were taken on an MKh 1310 instrument with an SVP-5 system for the direct introduction of the sample. The temperature of the evaporator ampul and of the ionizing chamber was 80-100°C, the ionizing voltage 50 V, and the collector current 60 μA. The relative error of the measurement of masses of the ions at R = 10,000 was $5 \cdot 10^{-6}$. Defocusing conditions: E, H = const, scanning of the accelerating voltage from 2.5 to 4.5 kV at the rate of 0.1 kV/sec, speed of the recorder chart 5 mm/sec.

For the conditions of separating the mixture of ecdysteroids on a high-performance liquid chromatograph, see [1].

22-Deoxyecdysterone. The methanolic extraction of 6 kg of the epigeal part of *S. nutans* collected by one of us in the environs of Ryazan' yielded 50.7 g of extractive substances [1]. Part of this total (18 g) was subjected to preliminary separation on a column of silica gel (chloroform—methanol (4:1) system), giving 2.12 g of a product consisting of a mixture of three phytoecdysteroids. The mixture was transferred to a preparative liquid chromatograph and, in addition to ecdysterone and polypodin B, 47 mg of 22-deoxyecdysterone (0.0023%) on the air-dry weight of the plant was isolated in the crystalline form with mp 241-242°C (from ethyl acetate—methanol): $[\alpha]_D$ +80,9±2° (*c* 0.42 ; methanol). Mass spectrum, *m/z* (%): 464 M+ (0,3), 446(1,4), 431(0,8), 428(35), **418(1,0)**, **413(16)**, 410(28), 400(2,5), 395(12), 385(0,7), 377(8,0), 367(7,0), 363(11), 361(1,0), 354(11), 345(43), 343(5,0), 327(54), 325(20), 320(20), 309(10), 302, 301(19), 300(35), 299(27), 250(30), 249(15), 145(20), 127(97), 109(100), 81(20), 69(54) . PMR spectrum (C_5D_5N, 100 MHz, HMDs, δ, ppm): 0.92 (3 H, at C-19, s); 0.99 (3 H at C-18, s); 1.22 (6 H at C-26 and C-27, s); 1.41 (3 H at C-21, s); 3.41 (H at C-9, m); 4.04 (2 H at C-2 and C-3, m); 6.02 (H at C-7, broadened singlet).

SUMMARY

A phytoecdysteroid — 22-deoxyecdysterone — has been isolated from the epigeal part of

Silene nutans L. It has been shown that it is 2β,3β,14α,20R,25-pentahydro-5β-cholest-7-en-6-one. Characteristic features of the mass spectrum of 22-deoxyecdysterone due to the absence of an oxygen function at C-22 have been discussed.

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CHROMATO-PHOTOCOLORIMETRIC DETERMINATION OF DIGOXIN

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Procedures have been developed for the quantitative determination of digoxin as such and in solutions for injection and tablets by a chromato-photocolorimetric method with the aid of which it is possible to obtain reliable results with adequate accuracy. The relative error of the determination does not exceed ±4.0%.

The quantitative determination of digoxin is carried out by a biological method [1]. Photocolorimetric, spectrophotometric, and chromatographic methods of analyzing digoxin have been described [5, 6, 10, 11]. It is reported in [2-4] that direct photocalorimetric and spectrophotometric methods are unsuitable for the investigation of the stability of preparations containing cardiac glycosides, and chromatographic methods have been developed mainly for the qualitative estimation of digoxin.

In view of this, the tasks of the present work were: 1) to study the chromatographic separation of digoxin from the foxglove and the breakdown products of digoxin; 2) to develop a procedure for the chromato-photocolorimetric determination of digoxin as such, in tablets, and in solutions; and 3) in order to establish the reliability of the results obtained, to compare them with those found by high-pressure liquid chromatography (HPLC) and by biological analysis.

EXPERIMENTAL

Standard digoxin, the ordinary substance, a solution for injection, and digoxin tablets were investigated. For the separation of digoxin, foxglove glycosides, and the products of its degradation we used ascending chromatography on Silufol UV-254 plates (Czechoslovakia) as the most readily available of the standard sorbents. The biological activity was determined on common frogs by the method of subcutaneous injection [1]. Analysis of digoxin by the HPLC method was performed by a procedure that we had developed previously [7].

We studied more than 20 solvent systems for the separation of foxglove glycosides. In the chloroform–acetone (1:1) solvent system, digoxin was separated from digitoxin and lantosides A, B, and C (Fig. 1).

Factors that have an unfavorable influence on the stability of drugs both in their production and during their storage are high temperatures, UV light, acidity or alkalinity of the glassware, and acidic or alkaline tablet fillers. In the choice of a system of solvents and reagents for revealing the zones of adsorption, digozin subjected to treatment with UV rays and to the action of alkalis and acids and also to high temperatures was

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