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STRUCTURE OF CYCLOHEXANONE DERIVATIVES FROM *DIGITALIS PURPUREA*

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Key Word Index—*Digitalis purpurea*; Scrophulariaceae; digitoxose; cyclohexanone derivatives; MS.

Abstract—Two cyclohexanone derivatives isolated from the growing tips of *Digitalis purpurea* were probably 8-oxabicyclo(3.3.1)-5-hydroxy-4-oxo-non-2-ene (*A*) and 2-hydroxy-2-(2-hydroxyethyl)-cyclohexanone (*B*).

INTRODUCTION

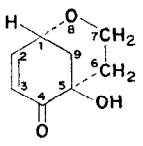
THE CARDIAC glycosides in *Digitalis purpurea* are neither uniformly distributed, nor is their composition the same in the different parts of the plant. It is well-established that the digitoxosides represent up to 90% of the total cardiac glycosides in the older parts of the plant, i.e. in second year leaves. On the other hand, the actively growing parts contain almost no digitoxosides, particularly when little or no chlorophyll is present.[1] Since we also failed to detect any free digitoxose, we may reasonably assume that the conditions for digitoxose biosynthesis are not fulfilled during the initial development stages of the plant or in absence of chlorophyll. Since it was considered possible that intermediates in the biosynthetic pathway of digitoxose might accumulate in these conditions our attention was directed to two new compounds, *A* and *B*, found at high levels when the digitoxosides were almost absent, and conversely, disappearing when the digitoxoside content increased. The isolation and structural elucidation of these compounds was therefore of considerable interest.

RESULTS AND DISCUSSION

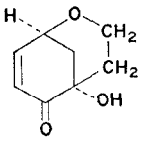
Compounds *A* and *B* were extracted from the growing tips of *Digitalis purpurea* with ethanol–water. The solids obtained on evaporation of the solvent were subjected to a trimethylsilylation according to the method of Sweeley.[2] The TMS ethers were purified on activated charcoal and hydrolyzed by boiling in aqueous methanol.[3] The resulting mixture of compounds was separated on a silica-gel column with the solvent EtOAc–*sec.* PrOH–H_2O (5:3:2) giving pure compound *A*. Compound *B*, still admixed with a small quantity of compound *A*, was again transformed to its TMS ether and further separated by preparative GLC. Compounds *A* and *B* show a number of common characteristics. They both possess a ketone or aldehyde function as shown by reactions with dinitrophenylhydrazine, hydroxylamine and potassium borohydride. However, the presence of an aldehyde group is precluded, since the NMR spectrum shows no signal for an aldehydic proton. The elemental

[1] LEMLI, J. (1961) *Verhandel. Koninkl. Vlaam. Acad. Geneesk. Belg.* **23**, 43.
[2] SWEELEY, C. C., BENTLEY, R., MAKITA, M. and WELLS, W. W. (1963) *J. Am. Chem. Soc.* **85**, 2497.
[3] WELLS, W. W., SWEELEY, C. C. and BENTLEY, R. (1964) *Biomedical Applications of Gas Chromatography* (Szymanski, H. A., ed.), p. 189, Plenum Press, New York.

compositions of both compounds are very similar: exact mass measurements revealed a difference of only four hydrogen atoms ($C_8H_{10}O_3$ for compound *A* vs $C_8H_{14}O_3$ for compound *B*).



(I)



(II)

Compound A

Enantiomeric structures (I) and (or) (II) are proposed for compound *A* on the basis of NMR and MS. In the NMR spectrum protons 2 and 3 appear as an *AX* quartet with the *A* part (proton 2) further split by the vicinal proton 1; irradiation of proton 1 reduces the signal of proton 2 to a doublet ($J_{2,3} = 10$ Hz). Proton 2 (6·67 δ) is found at a lower field than proton 3 (5·86 δ), as a result of the deshielding effect of the carbonyl group. This observation indicates that these two protons are part of an α,β unsaturated ketone structure. The UV spectrum of compound *A* with a maximum at 222 nm also conforms to a cyclohexenone structure unsubstituted at the C-2 and C-3 (double bond) positions.[4] The sextet centered at 4·13 δ was assigned to proton 1. This sextet is due to the already mentioned coupling of protons 1 and 2 resulting in a doublet, which is further split by the two methylene protons at C-9. Irradiation of the methylene protons caused the sextet to collapse to a doublet ($J_{1,2} = 1{\cdot}8$ Hz) The methylene protons (9,9′) at C-9 are not equivalent. They were found as the *AB* part of an *ABX* system with proton 1 as the *X* part ($J_{1,9} = J_{1,9'} = 4{\cdot}5$ Hz). Since the shift positions of protons 9 and 9′ approach each other very closely (2·54 and 2·64 δ respectively), overlapping occurs.[5] From the 8 lines expected for the *AB* part, the four outer lines are obscured by noise; the two inner lines coincide so that the absorption signal appears as a triplet. Decoupling of the protons 9 and 9′ from proton 1 leaves the two interior lines of the *AB* pattern. The same explanation holds true for the protons 7 and 7′ (ABX_2 system). Here again, only the two interior lines of the *AB* part are perceived, each being further split into a triplet by the two protons at C-6 ($J_{7,6} = J_{7',6} = 7{\cdot}2$ Hz); *ca.* δ for 7 and 7′ = 3·81 and 3·91). The multiplet from the methylene protons at C-6 (*ca.* 2·22 δ) is partially overlapped by the signal from acetone-d_5, present in trace amounts in the acetone-d_6 solvent. The singlet at 2·88 δ was assigned to the hydroxyl proton since it is removed by deuterium oxide.

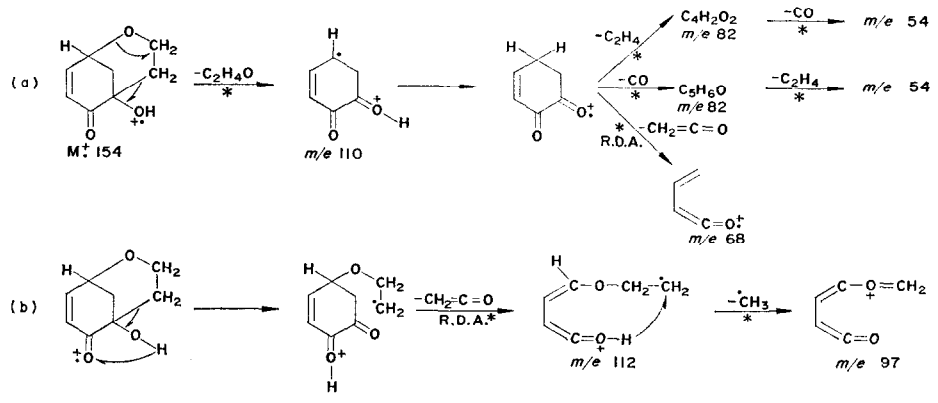
The MS of compound *A* is most easily interpreted when the positive charge of the molecular ion is located either on the tertiary alcohol function, or on the carbonyl function (Scheme 1). In the former case the ether bridge can be lost, probably as a molecule of ethylene oxide,[6a] to give the ion *m/e* 110. After the rearrangement of a hydrogen atom, the resulting cyclohexenedione ion loses ethylene, carbon monoxide or ketene by separate fragmentation pathways (Scheme 1a). The alternative pathway involves a retro-Diels–Alder elimination of ketene from the molecular ion (Scheme 1b). The resulting *m/e* 112 ion further loses a methyl radical.[6b,c]

[4] SCOTT, A. I. (1964) *Interpretation of Ultraviolet Spectra of Natural Products*, p. 63, Pergamon Press, Oxford.

[5] BOSE, A. K. (1965) *Interpretive Spectroscopy* (FREEMAN, S. K., ed.), p. 223, Reinhold, New York.

[6] (a) BUDZIKIEWICZ, H., DJERASSI, C. and WILLIAMS, D. H. (1967) *Mass Spectrometry of Organic Compounds* p. 151, Holden-Day, San Francisco; (b) *ibid.* p. 67; (c) *ibid.* p. 143.

The molecular ion (*m/e* 226) of the TMS derivative of compound *A* corresponds with the presence of a single hydroxyl group. In close analogy with the spectrum of the parent compound, losses of ketene (*m/e* 184), ketene and methyl (*m/e* 169) are observed.[7] The presence of a TMS group is revealed by the loss of a methyl radical (*m/e* 211) and of trimethylsilanol (*m/e* 136).

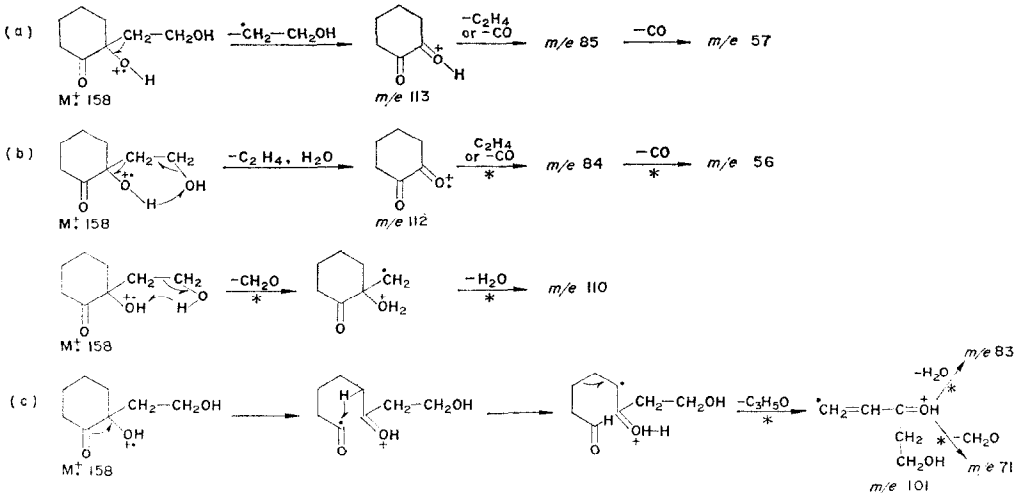


SCHEME 1.

Compound B

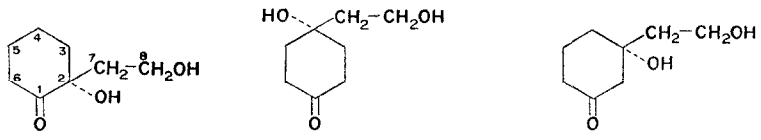
A structural relationship between compounds *A* and *B* was presumed on the basis of the following observations: their similar elemental compositions and the occurrence of a ketone group in both compounds; furthermore, they are always found together in the same parts of the plant and they might well be involved in the same biological pathway, In addition to the fact that compound *B* possesses four hydrogen atoms more than compound *A*, it also contains two hydroxyl functions as shown by the introduction of two trimethylsilyl groups in the TMS derivative. Saturation of the –CH=CH– double bond and reductive opening of the ether bridge in compound *A* would account for the presence of four additional hydrogen atoms and two hydroxyl groups in compound *B*. The resulting structure is in agreement with MS evidence. Several fragmentations confirm the presence of a $-CH_2-CH_2OH$ side chain localized on a carbon atom bearing a hydroxyl group. Elimination of the side chain via simple α-cleavage is triggered by the tertiary hydroxyl function and gives rise to the abundant ion *m/e* 113 (Scheme 2a). The spectrum of TMS derivative of compound *B* shows an ion *m/e* 185, differing from the *m/e* 113 analog by the presence of one TMS group. This finding not only confirms that compound *B* contains one ring hydroxyl group, but also reveals the side chain location of the other hydroxyl group. The primary character of the latter hydroxyl group is indicated by the presence of a characteristic *m/e* 31 ion in the spectrum of the parent compound and of an analogous *m/e* 103 ion in the spectrum of the TMS derivative. These ions can be represented as $CH_2{=}OH^+$ and $CH_2{=}O^+$–TMS, respectively. Further confirmation of the relative positions of the two hydroxyl groups is given by two other fragmentations which require the transfer of one hydroxyl hydrogen atom to the other hydroxyl group and which must proceed via a 6-membered transition state (Scheme 2b). Migration of a hydrogen atom from the tertiary to the primary hydroxyl group initiates the elimination of ethylene and water, producing the ion *m/e* 112. The alternative pathway

consists of a hydrogen transfer from the primary to the tertiary hydroxyl group and gives rise to consecutive losses of formaldehyde (*m/e* 128) and water (*m/e* 110). These fragmentations are confirmed by the appropriate metastable ions. Both reactions have counterparts in the fragmentation of the C-17 side chain of cortisol.[8] The most prominent fragment ions



SCHEME 2.

(*m/e* 55 and *m/e* 101) in the spectrum of compound *B* are characteristic of the presence of a 2-hydroxy-cyclohexanone structure[9] (Scheme 2c). The *m/e* 101 peak shifts to *m/e* 245 in the spectrum of the TMS derivative of compound *B*. This shift of 144 m.u. proves that the $C_5H_9O_2$ species (*m/e* 101) present in the spectrum of the parent compound, retains the two hydroxyl groups and that the moiety containing the carbonyl group was lost. The structures (III), (IV) and (V) and their enantiomers can account for the hitherto described fragmentation reactions. For structure (V), however, in addition to the *m/e* 55 and *m/e* 101 ions, one expects to find a fragment ion with *m/e* 115 consisting of the C-1, C-2 and C-3 carbon atoms plus side chain, and retaining the 3 oxygen atoms. Since such an ion was not present in the spectrum of compound *B*, only structures (III) and (IV) are left on the basis of MS evidence.



However, if there is a biogenetic relationship between compounds *A* and *B*, structure (III) is to be preferred. Whether these compounds have any direct connection with digitoxose remains to be seen.

[7] DRAFFON, G. H., STILWELL, R. N. and MCCLOSKEY, J. A. (1968) *Org. Mass Spectrom.* **1**, 669.
[8] GENARD, P., PALEM-VLIERS, M., CONINX, P., MARGOULIES, M., COMPERNOLLE, F. and VANDEWALLE, M. (1968) *Steroids* **12**(6), 763.
[9] SILVERSTEIN, R. M. and BASSLER, G. C. (1967) *Spectrometric Identification of Organic Compounds*, p. 20, Wiley, New York.

EXPERIMENTAL

Extraction and isolation of compounds A *and* B. Freeze-dried and finely ground growing tips (5 g) were successively extracted with 50 ml EtOH 80%, 50 ml EtOH 70% and 50 ml H_2O. The solids obtained on evaporation (45°, *in vacuo*) of the solvent were dissolved in pyridine and compounds *A* and *B*, together with the carbohydrates present, were converted into their TMS ethers.[2] An appropriate purification of the TMS ethers was obtained by pouring the solution on a small column of activated charcoal (5 g). The TMS ethers were eluted with 20 ml light petrol. and hydrolyzed by boiling for 2 hr in 50% aq. MeOH.[3] After evaporation of the solvent, the residue was taken up in a few ml of H_2O and adsorbed on a silica-gel (0·2–0·05 mm) column (50 g). The column was developed with EtOAc–*sec.* PrOH–H_2O (5:3:2). The effluent was collected in 20 ml fractions and concentration of fractions 3 and 4 afforded 48 mg of pure compound *A*. Compound *B* (fractions 5–7) was again transformed into its TMS ether and further separated by GLC using the 104/64 Pye Chromatograph fitted with a manual preparative kit. GLC conditions were as follows: coiled glass column (4·6 m by 0·95 cm o.d.) was packed with equal parts of 1% HI-EFF 8 BP on 60–80 mesh Gaschrom Q and 3% OV 17 on 60–80 mesh Gaschrom Q (Applied Science Laboratories); flow rate of the Argon carrier gas was 70 ml/min; column oven was programmed from 160° to 210° at a rate of increase of 3°/min. After hydrolysis of the TMS ether, we obtained about 10 mg of pure compound *B*.

NMR spectrometry. NMR spectra were recorded on a Varian HA 60 (60 MHz) spectrometer. Spectra were run in acetone-d_6 with tetramethylsilane as internal standard.

Mass spectra were recorded on an A.E.I. MS 12 spectrometer. The samples were inserted on a direct probe. The temperature of the ion source was 100–150°. The spectra were run at an ionizing voltage of 70 eV and an accelerating voltage of 8 kV. The major peaks observed in the MS of compound *A* were as follows: *m/e*, 154, 136, 112, 110, 107, 97, 82, 68, 54, 41; A%, 21, 21, 35, 87, 24, 24, 100, 49, 25, 10 with metastable peaks at *m/e* (m_2^2/m_1) 120·1 ($136^2/154$); 84·0 ($97^2/112$); 81·6 ($112^2/154$); 78·7 ($110^2/154$); 64·3 ($79^2/97$); 61·1 ($82^2/110$); 49·4 ($82^2/136$); 42·1 ($68^2/110$); 35·5 ($54^2/82$). The major peaks observed in the MS of compound *B* were as follows: *m/e*, 158, 140, 128, 113, 112, 110, 101, 95, 85, 84, 83, 73, 71, 70, 67, 57, 55, 43, A%, 58, 42, 15, 60, 55, 15, 100, 28, 60, 31, 68, 32, 62, 57, 41, 73, 100, 93 with metastable peaks at *m/e* (m_2^2/m_1) 124·1 ($140^2/158$); 106·3 ($122^2/140$); 103·8 ($128^2/158$); 94·5 ($110^2/128$); 68·2 ($83^2/101$); 64·6 ($101^2/158$); 63·1 ($84^2/112$); 37·2 ($56^2/84$). The exact mass of some prominent peaks was determined on 'expanded scale records' by linear interpolation between unknown and reference peaks, differing no more than 3 m.u. The photosensitive paper was calibrated by means of the known difference between C^{12} and C^{13} isotopes. The exact mass of the following peaks was determined: Compound *A*: *m/e* 154 (molecular formula $C_8H_{10}O_3$), 112 ($C_6H_8O_2$), 110 ($C_6H_6O_2$), 97 ($C_5H_5O_2$), 68 (C_4H_4O), 82 (85%) ($C_4H_2O_2$), 82 (15%) (C_5H_6O). Compound *B*: *m/e* 158 (molecular formula $C_8H_{14}O_3$), 113 ($C_6H_9O_2$), 112 ($C_6H_8O_2$), 101 ($C_5H_9O_2$).

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