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HIRUNDIGENIN AND ANHYDROHIRUNDIGENIN, TWO NATURAL 15-OXASTEROIDS OF PLANT ORIGIN. CHEMICAL AND X-RAY INVESTIGATION.

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Cynanchum vincetoxicum (L.) Pers. or Vincetoxicum hirundinaria Medikus are the valid names (1) for the sole representative of the family of Asclepiadaceae growing wild in Central Europe. It is a polymorphous species (usually named Vincetoxicum officinale Mönch). The roots which were formerly used as medicinal drugs (Radix Vincetoxici), contain a mixture of glycosides (2,3,4,5, 6,7). Dried roots collected in northern Italy, yielded (8) ca. 5% crude glycosides. After mild acidic hydrolysis we isolated three new aglycones: hirundigenin ($C_{21}H_{30}O_5$){1}, anhydrohirundigenin ($C_{21}H_{28}O_4$){3} and vincetogenin ($C_{21}H_{28}O_6$). We give here a preliminary report on the structure of {1} and {2}, which are both 15-oxasteroids, of a type to our knowledge not previously encountered in natural products. Chemical reactions together with UV.-, IR.-, NMR.- and mass spectra could be accounted for by a number of possible structures of which {1} and {3} were the most probable. Although the chemical evidence alone was not conclusive, it was possible to establish these structures by an X-ray diffraction analysis of {5}. We give here a brief report of the results of both ways of approach. Full details of the chemical investigations will be published in Helv. Chim. Acta and the X-ray work in Acta Crystallographica.

X-Ray Analysis. To facilitate the analysis a heavy atom was introduced into the structure by preparing the p-bromobenzoate of anhydrohirundigenin {5}.

Crystal Data. $C_{28}H_{31}O_5Br$ M.P. = 244-248°(dec.). Needle shaped crystals elongated b. Monoclinic; a = 9.635±4, b = 5.868±4, c = 22.136±7Å; β = 97.15±3°. Space group $P2_1$ from absences. F(000) = 548. The intensities were measured on a four-circle automatic diffractometer in the 2θ scan mode, using copper radiation (λ = 1.5418Å). Of the 2516 points explored 2412 had significant intensities. No corrections were made for absorption or extinction.

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The bromine positions, deduced from a 3-dimensional Patterson synthesis were used to prepare the first 3-dimensional Fourier distribution. The map was poorly resolved, complicated by pseudosymmetry, and numerous attempts to solve it were unsuccessful. A more promising approach was the Woolfson-Sim (9, 10) method of weighting the observed structure amplitudes by a factor which reflects the probability that the difference between the phase angle for the complete structure and the phase angle based on atoms in known positions is small.

The Fourier map calculated with such weighted observed structure factors and phase angles derived from the bromine positions showed a marked improvement in resolution and it was possible to locate the p-bromobenzoate group. A second "weighted" Fourier map phased on this group showed the position of most of the atoms in the steroid skeleton.

The direct Fourier map was still complicated by pseudosymmetry and the remaining seven atoms were located from a difference synthesis. The marked pseudosymmetry of the map was accounted for by the positions of eleven of the light atoms, whose y-coordinates were all within ± 0.2Å of the y-coordinate of bromine, set at 0.5. The last two oxygen atoms were assigned on basis of relative peak heights to positions which also appeared chemically reasonable. The structure was refined through two cycles of full-matrix least-squares calculations using isotropic thermal parameters and three cycles of block-diagonal calculations with only bromine vibrating anisotropically. At this stage the assignment of the oxygen atoms in the anhydrohirundigenin molecule was tested by giving them scattering factors appropriate to carbon. On refinement, the temperature factors of these atoms decreased markedly while those of the other atoms remained essentially unchanged, thus confirming the assignment.

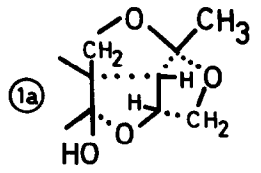
Refinement continued with full matrix calculations. All hydrogen atoms were located from difference maps and included, but not refined, in the final cycles of calculations. The final reliability factor was 4.7% with all atoms, other than hydrogens, treated anisotropically.

The bond lengths and angles based on the final atomic positions for anhydrohirundigenin are shown in Fig. 1a, b. The standard deviation of bond lengths between carbon-carbon or carbon-oxygen is between 0.004 - 0.008Å and that of the bond angles between 0.2° - 0.5°. The bond length and angles in the p-bromobenzoate group are within the normal range found in similar structures.

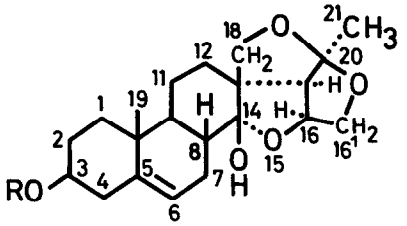
The stereochemistry of the molecule is illustrated in Fig. 2. The orientation of the hydrogen atoms at C17 is α and thus corresponds to formula 1b, which fits in well with the proposed biogenetic pathway. Ring A is in the normal chair form, ring B is intermediate between a sofa and a half chair and C is a sofa, and the three five-membered rings are in the envelope conformation with C(18), O(3) and O(4) forming the flaps of the three envelopes respectively. A full conformational analysis is being undertaken and the results will be published in the main crystallographic paper.

Chemical Evidence. All compounds for which empirical formulae are quoted gave correct analytical results in combustion analysis or mass spectra or both.

Hirundigenin {1} has only one isolated double bond ($\lambda_{max}^{alk} < 195$ nm, $\varepsilon \sim 8320$ at 195 nm), two hydroxyl groups of which only one is acetylated at 25°; carbonyl groups are absent (IR.). Three oxygen atoms must therefore be present as ether groups. The absence of methoxy or ethoxy groups and any evidence for peroxides, dioxalan or dioxan rings together with the empirical formula shows that the compound contains only three carbocyclic rings.



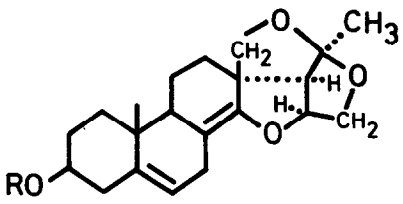
(1a)



(1) (R=H) Hirundigenin (KST-5)
F. 190-196° [-44,8 Chf] $C_{21}H_{30}O_5$

(2) (R=Ac) (KST-10)
F. 168-178° [-41,8 Chf] $C_{23}H_{32}O_6$

Δ

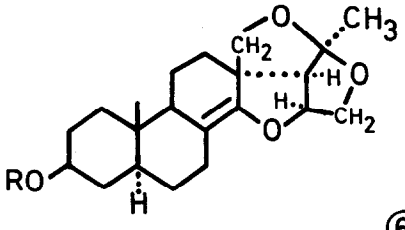


(3) (R=H) Anhydrohirundigenin (KST-1)
F. 212-217° [-25,8 An] [-27,4 Chf] $C_{21}H_{28}O_4$

(4) (R=Ac) (KST-3)
F. 156-158° [-30,1 An] $C_{23}H_{30}O_5$

(5) (R=p-Bromobenzoyl-) (KST-40)
F. 244-248° [+0,6 Chf] $C_{28}H_{31}O_5Br$

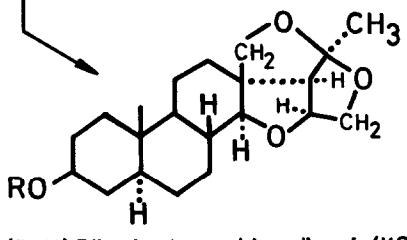
H_2+Pt



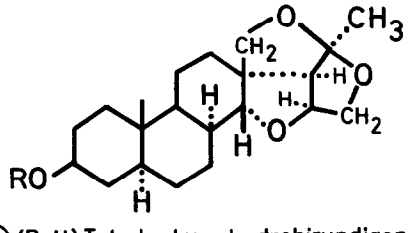
(6) (R=H) Dihydroanhydrohirundigenin (KST-4)
F. 192-205° [+91,5 Chf] $C_{21}H_{30}O_4$

(7) (R=Ac) (KST-8)
F. 178-182° [+64,9 Chf] $C_{23}H_{32}O_5$

(1) H_2+Pt



(6) H_2+Pt



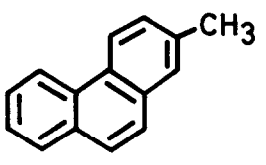
(8) (R=H) Dihydrodesoxyhirundigenin (KST-7)
F. 224-226° [-26,1 Chf] $C_{26}H_{32}O_7$

(9) (R=Ac) (KST-17) F. 190-193° [-34,9 Chf] $C_{23}H_{34}O_5$

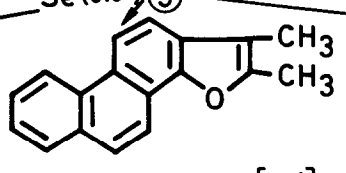
(10) (R=H) Tetrahydroanhydrohirundigenin (KST-6) F. 175-177° [+61,4 Chf] $C_{21}H_{32}O_4$

(11) (R=Ac) (KST-16) F. 166-170° [+41,7 Chf] $C_{23}H_{34}O_5$

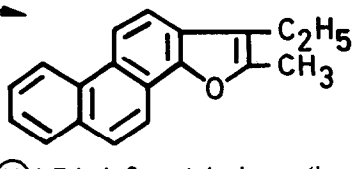
Se (310°) (3)



(12) 2-Methylphenanthrene
F. 55-56°



(13) 1,2-Dimethyl-phenanthro-[1,2-b]-furan (KST-29) F. 154-156°
$C_{18}H_{14}O$

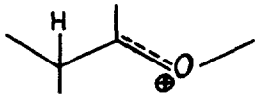


(14) 1-Ethyl-2-metyl-phenanthro-[1,2-b]-furan (KST-30)
F. 82-85° $C_{19}H_{16}O$

Hirundigenin is smoothly transformed into anhydrohirundigenin {3} by sublimation in vacuo. The latter shows strong additional absorption in the short wave UV. at about 202 nm ($\varepsilon \sim 11{,}500$), indicative of a fully substituted double bond. This is supported by the NMR spectra of {1} and {3} and their mono-O-acetyl derivatives, which all show only one signal attributable to a vinyl proton. The IR.-spectrum of {3} shows a strong band at 1715 cm^{-1} (in KBr), i.e. in the region of C=O vibration (only with reduced intensity). Since anhydrohirundigenin is stable to $LiAlH_4$ in boiling tetrahydrofurane, it cannot contain a carbonyl group. The conspicuous IR.- band must be due to enolether $\rangle C{=}C\langle_{O-}$ absorption, usually found at longer wave length[1]. Hydrogenation of anhydrohirundigenin under mild conditions yields the dihydrocompound {6} in which the UV.- and IR.-absorption is still present. Under more vigorous conditions two molecules of H_2 are slowly taken up and two isomers are formed to which we assign formulae {8} and {10}. The isomer {8} is obtained in practically pure state by hydrogenation of hirundigenin {1}. This reaction proceeds very quickly and involves hydrogenolytic loss of the tertiary hydroxyl group. {8} and {10} contain no double bonds or carbonyl groups and only one hydroxyl group as both give mono-O-acetyl derivatives without OH-absorption in the IR. All these compounds must therefore still contain three ether oxygens and 3 carbon rings.

To establish the ring skeleton of these compounds the remaining material of anhydrohirundigenin (971 mgs) was dehydrogenated with selenium at 310° for 24 hours. From the mixture of neutral products three crystalline components {12}, {13} and {14} were isolated together with two oily compounds of unknown structure. {12} was identified as 2-methylphenanthrene, a normal dehydrogenation product of many steroids (11, 12). The two phenanthrofurans {13} and {14} are new compounds. Their probable structure was deduced from UV.-, IR.-, NMR.- and mass spectra and proved by synthesis.

Further chemical results together with NMR.-, IR.-, UV.- and mass spectra strongly favour formula {1} for hirundigenin, {1a} could not be excluded but would involve much steric strain. According to IR.- and NMR.- spectra the hydroxy group in O-acetylhirundigenin {2} is strongly bridged compatible only with a β-position. In the NMR.-spectra of the fully hydrogenated compounds {8} and {10} new signals turn up as doublets at 3.07 and 4.06 ppm which we assign to the 14-H. The high coupling constants (J = 10 and 9 Hz. respectively) are indicative of a 8,14-trans configuration. The stereochemistry of {8} - {11} are given on the assumption (not necessarily correct) that hirundigenin {1} possesses the normal 8β-configuration. From this we conclude that hydrogenolysis of {1} → {8} takes place with inversion of configuration at C-14. The thermic conversion of {1} → {3} would represent a cis-elimination. The hydrogenation of the 8,14 double bond in acetic acid must follow an unusual path, perhaps via carboxonium ions {15} and {16} (13) of which {15} adds hydrogen from the α- and {16} from the β-side.



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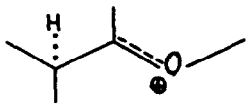

{16}

1) Dr. B. S. Gallagher, Institute for Steroid Research, Montefiori Hospital and Medical Center, Bronx, N.Y. has kindly measured the IR.-absorption of {3} and {6} and also concluded that the band must be due to a perturbed $\rangle C{=}C\langle_{O-}$ absorption.

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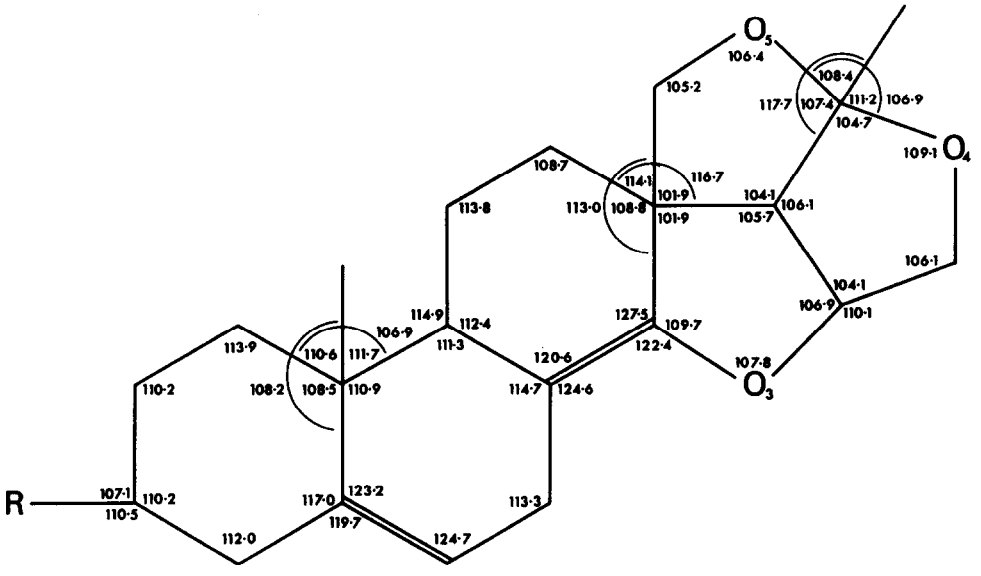

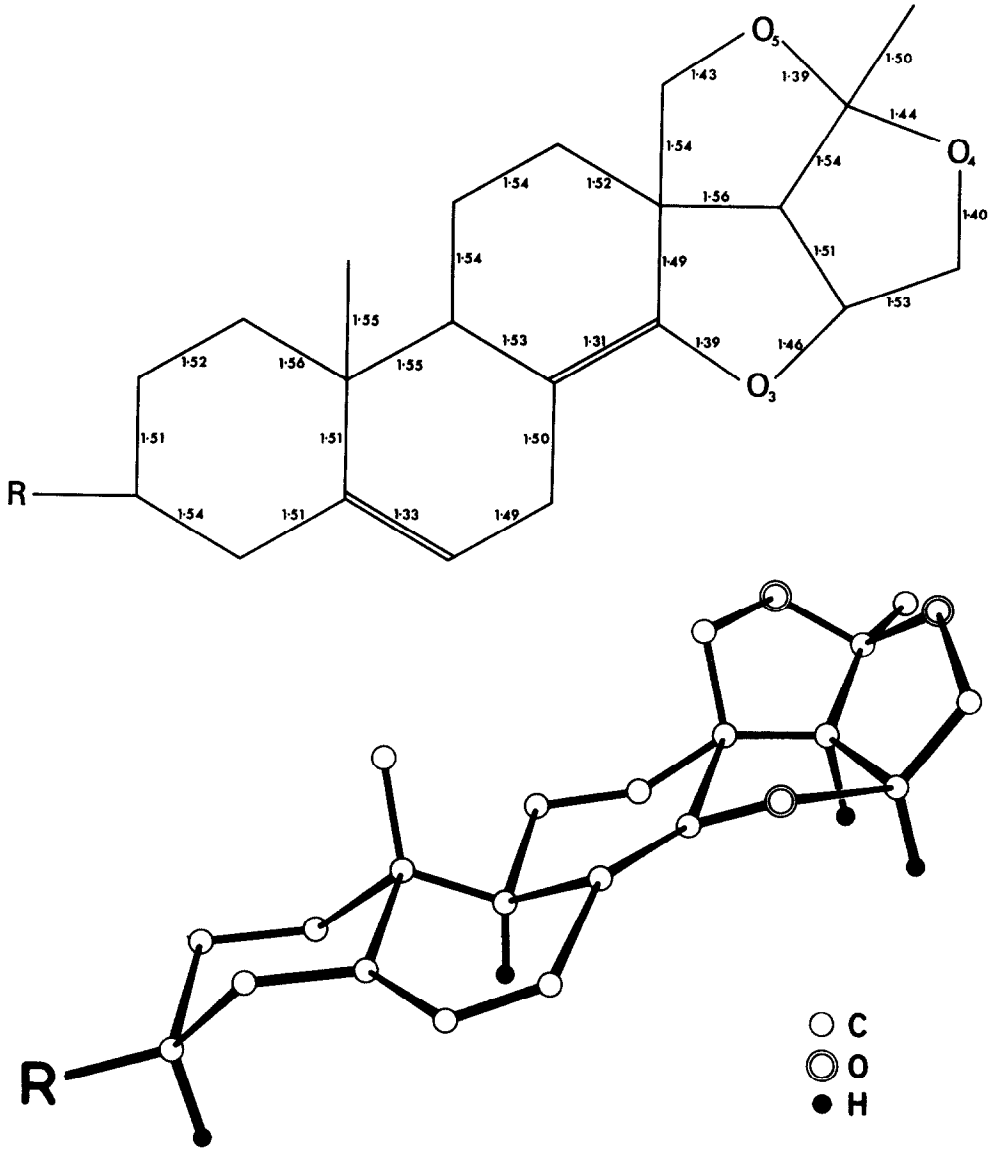
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