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THE STRUCTURE OF SILYBIN (SILYBUM SUBSTANCE E_6), THE FIRST FLAVONOLIGNAN

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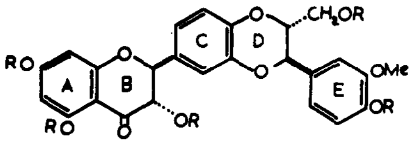
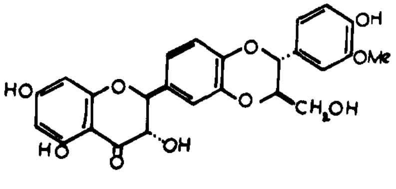
The substance silybin[1,2], isolated from the fruits of Silybum marianum Gaertn., has been under investigation for a number of years. Initial studies[3] based on colour tests and U.V. and I.R. spectra indicated that the substance was a flavanonol of an unknown kind, and a recent structure proposed by Hänsel and Schöpflin[4] indicated that it was a 3' -methyltaxifolin substituted at the 4' -position by a chromanol moiety.

It was however difficult to reconcile this formula with the reported mass spectra (in particular) and a further examination of the evidence was felt to be necessary. Detailed analysis of the 100Mc. P.M.R. spectrum of E_6 and its derivatives, together with a new interpretation of the mass spectra, lead to the proposal that silybin, is, in fact, represented by (1a) or its isomer (1').

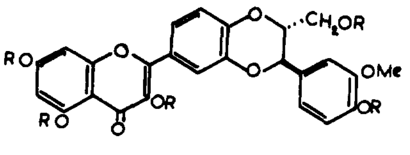
The substances examined were silybin (1a), $C_{25}H_{22}O_{10}$[4], the acetate (1b), $C_{35}H_{32}O_{15}$, the flavonol (2a), $C_{25}H_{20}O_{10}$, derived from silybin by dehydrogenation and known as E_6F, E_6F acetate, $C_{35}H_{30}O_{15}$ (2b) and methyl E_6F, $C_{30}H_{30}O_{10}$, (2c).



1 (a) R=H
(b) R=Ac

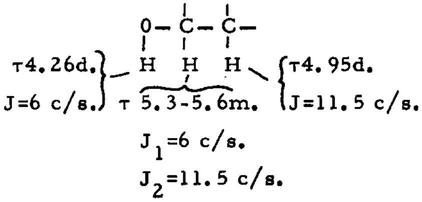


(1')

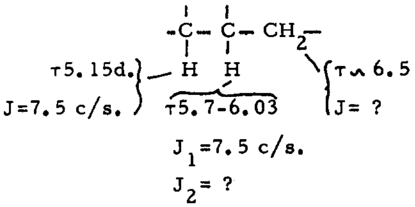


2 (a) R=H
(b) R=Ac
(c) R=Me

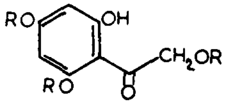
The 100Mc. P.M.R. spectrum of E_6 itself (run in hexadeuteriodimethyl sulphoxide) was of interest. In the aromatic region there were nine protons, one of which however was lost on addition of D_2O. A readily identifiable pair of doublets was clear at τ4.13 and τ4.18 (J = 2 c/s.), indicative of two protons, meta coupled, each between two oxygen atoms on a benzene ring. Three hydroxyl groups were present at τ1.94, -0.5 to -1.0, and -1.81. Attention was turned to the complicated aliphatic region and one clear doublet (1H) at τ4.26 (J = 6 c/s.) was saturated, causing only one change in the spectrum, a complex multiplet at τ5.3 - 5.6 collapsed to a sharp doublet (J = 11.5 c/s.). This was shown to be one partner of an AB pair, the other proton of which was centred at τ4.95 (J = 11.5 c/s.), by further saturation experiments. An ABC system is therefore present, and as addition of D_2O caused the doublet at τ4.26 to disappear, and changed the other protons to sharp doublets, it is clear that the system is as shown. That the hydroxyl proton is exchanging slowly on the N.M.R. time scale is due to the solvent used.



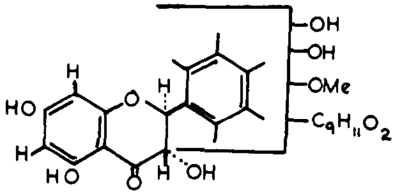
Similar experiments served to break down the other aliphatic protons into the system shown, though the $-CH_2$ group could not be clearly seen. One aromatic methoxy grouping was present.



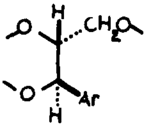
In view of the reported isolation of (3b) by the action of base on (2c), our own characterisation of (3a) as a product from the action of aluminium chloride on silybin, and the P.M.R. results reported, part structure (4) may be regarded as established for silybin.



3 (a) R=H
(b) R=Me



(4)



(5)

As derivatives of E_6F lack the complications of the flavanonol system, more information regarding the unknown portion should be available. The known stability of the flavonol ring under electron impact, as compared with the flavanonol ring[5], should also lead to more information regarding the C_9 fragment unaccounted for.

The P.M.R. spectra of (2b) and (2c) are shown in the Table.

In the E_6F system there are only four aliphatic protons. On acetylation two of the protons move downfield by ∿τ0.5, indicative of the presence of a primary hydroxyl grouping in E_6F itself. The two hydrogen atoms of the methyleneoxy group however are quite distinct, showing the group is next to an asymmetric centre, $R_1R_2CH.CH_2OX$.

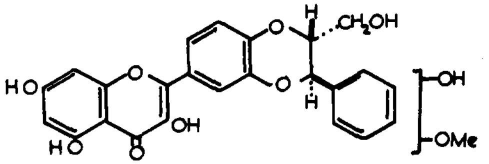
TABLE*

Protons	2b	2c
Aromatic	τ2.4-3.2 (8H)	τ2.2-3.7 (8H)
Aliphatic	τ5.03d. (J=7.5 c/s)(1H) τ5.5-5.8m. (2H) τ5.85-∿6.2 (1H)	τ4.95d. (J=8 c/s)(1H) τ5.7-5.9m. (1H) τ6.25-6.45q. (1H) τ6.5-∿6.7 (1H)
Methoxy	τ5.15s (3H)	τ6.0-6.24 (15H) τ6.62 (3H)
Acetyl	τ7.59 (3H) τ7.68 (9H) τ7.95 (3H)	
Total	30 H	30 H

*Spectra run in $CDCl_3$/TMS as internal standard.

This is similar to the situation in sitsirikine and its acetate in which the methyleneoxy grouping is next to a carbon bearing a hydrogen atom, an electron withdrawing group and an aliphatic carbon atom[6]. The pattern for the two protons, as well as the shift on acetylation is strikingly similar in the two compounds.

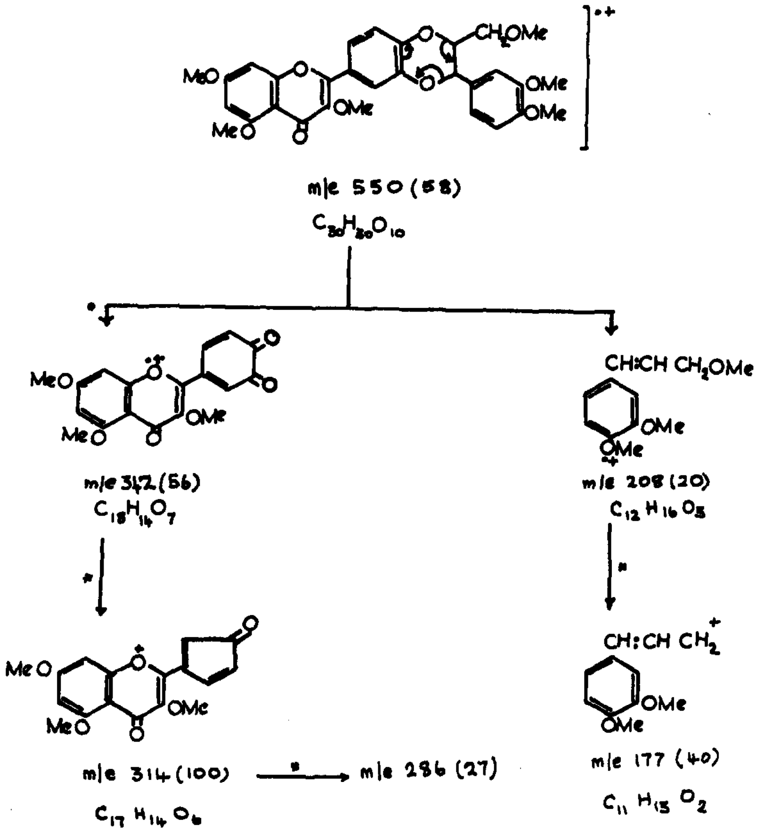
In (2c), all the aliphatic protons may be discerned separately. That at τ4.95d is coupled with the multiplet at τ5.7-5.9, which on irradiation at τ4.95, becomes a very broad singlet. Saturation at τ5.85 caused the doublet at τ4.95 to collapse to a singlet, whilst the quartet at τ6.25-6.45 became a doublet (J=8 c/s). Irradiation at τ6.35 caused the multiplet at τ5.7-5.9 to become a rough doublet. Taken together with the τ values and the J values these results show presence of the system (5); similar experiments with (2b) confirm these conclusions. If this is added to part structure (4), and the previous evidence[3] that silybin is a flavanonol bearing two oxygen atoms at C-3' and C-4' is taken into account, the part structure (6) must be written for E_6F, the



(6)

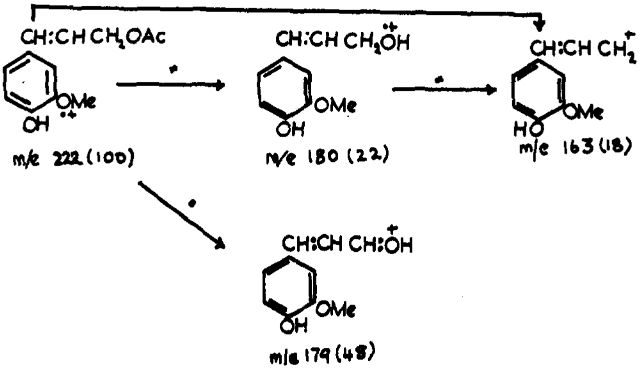
1,4-dioxane ring adopting a half-chair form with the two substituents in quasi-equatorial positions. In view of the known participation of coniferyl alcohol as a C_9 unit in lignin formation[7], we prefer to place the hydroxy and methoxy groups on ring E as in (2), this leading to structures (1a) or (1') for silybin itself.

It is now possible to both rationalise the mass spectra and support structure (1). The mass spectrum of methyl E_6F has significant peaks at m/e 555.181988 ($C_{30}H_{30}O_{10}$), 342.074139 ($C_{18}H_{14}O_7$), 314.079351 ($C_{17}H_{14}O_6$), 286.084116 ($C_{16}H_{14}O_5$), 208.110538 ($C_{12}H_{16}O_3$) and 177.09183 ($C_{11}H_{13}O_2$). Scheme 1 provides reasonable pathways to these fragments, all the transitions shown being supported by metastable ions. In addition the breakdowns show that two methoxyl groups are attached to ring E in methyl E_6F. For E_6F itself this point is further made by the appearance of large peaks at m/e 180, 162, 138 and 124, showing that one hydroxyl and one methoxyl group are on ring E. The mass spectrum of E_6 acetate is richly instructive, as despite the facile losses of ketene and acyloxy radical open to the molecule, it is still the reverse Diels-Alder reaction in



Scheme 1.

the 1,4-dioxane ring that provides the base peak at m/e 222.088646 ($C_{12}H_{14}O_4$).



Scheme 2.

The fragmentations associated with the other portion of the molecule are clearly defined, but will be discussed elsewhere.

It is clear that silybin is a 1,4-dioxane produced by the oxidative combination of a flavonoid unit (taxifolin) and coniferyl alcohol. The spectroscopic data however do not distinguish whether the union has taken place to yield (la) or (l'), although structures based on (la) have, for convenience been used throughout the present account. Another ambiguity lies in the stereochemistry of silybin, for although the relative configurations of the substituents on ring B and ring D are known separately, these have not been correlated.

Silybin is the first member of a new class of compound, that may be of some importance, and for which the generic name 'flavonolignan' is proposed. The possible biogenesis and role of such compounds will be discussed, together with much other chemical data in a full paper. At this stage it suffices to note that de Stevens and Nord[8], have implicated flavanones as possible constitutive elements in bagasse native lignin, the mode of linkage of the flavanone and C_9 units being unknown. Silybin provides the first clear example of such a linkage.

We wish to express our thanks to Mr. R. Warren for the 100Mc. spectra.

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