Constituents of *Silybum marianum*. Structure of Isosilybin and Stereochemistry of Silybin

By ALBERTO ARNONE, LUCIO MERLINI,† and ANTONIO ZANAROTTI

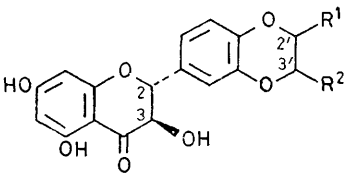
(*Istituto di Chimica, Politecnico, Centro C.N.R. per le Sostanze Organiche Naturali, I-20133 Milano, Italy*)

Summary The isolation and structural elucidation of the flavolignan isosilybin is reported; both silybin and isosilybin are shown to be mixtures of diastereoisomers.

THE chemistry of the natural substances contained in the fruits of *Silybum marianum* Gaertn. (Mariendistel)[1] has been the subject of numerous investigations, since the discovery of the antihepatotoxic activity of one of the constituents, the flavolignan silybin (**1**).[2] After different structural proposals, degradative work,[3] and synthesis of dehydrosilybin pentamethyl ether,[4] the structure (**1**) for silybin was established in 1975.

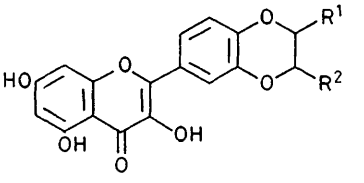
Besides silybin, the crude extract of the plant contains two other products, silychristin and silydianin,[5] apparently derived from different coupling modes of dihydroquercetin and coniferyl alcohol.[6] However, Wagner *et al.*[7] reported in 1974 the presence of another constituent, tentatively called isosilybin, which was not isolated, and which appears just after silybin on t.l.c. of the crude extract.‡

Careful preparative t.l.c. of the crude extract of *Silybum marianum* (silica gel, chloroform–EtOAc–acetone–HCO_2H, 8:1:1:0·1) gave pure isosilybin, m.p. 239—241 °C (from EtOAc). Structural (i.r., u.v., mass, and ^1H and ^{13}C n.m.r.) analysis indicated that isosilybin is identical to the isomer obtained in our biomimetic synthesis of silybin[9] and that it is similar to silybin,§ thus indicating that it is a regio- or diastereo-isomer of silybin. As the coupling constant values are 8 Hz for 2- and 3-H (2′- and 3′-H) in both the flavanonol and benzodioxan rings in the n.m.r. spectrum of isosilybin, both the pairs of ring substituents must be *trans*[10] as in silybin. The configurational identity of natural and synthetic isosilybin (as shown by comparison of c.d. curves) establishes the 2*R*,3*R* configuration for C-2 and C-3 of the flavanonol ring, which is also consistent with the similarity between the c.d. spectra of silybin and isosilybin; c.d.: 255 ($\Delta\epsilon$ + 2·84), 295 (−14·70), and 330 nm (+3·46) for isosilybin in dioxan (*c* 28·4 × 10^{-3}). Conclusive evidence for the structure (**2**), *i.e.* for a regioisomer of silybin at the benzodioxan ring, came from mild dehydrogenation of isosilybin in boiling pyridine,[11] which afforded quantitatively dehydroisosilybin (**4**). The structure of (**4**), from mass and n.m.r. spectra, is similar to that of the corresponding compound (**3**) obtained from silybin.[3] However, both dehydro derivatives clearly show different behaviour on t.l.c. This non-identity eliminates the possibility of diastereoisomerism at C-2′ and C-3′ between (**1**) and (**2**), which would lead to identical dehydro derivatives; thus (**2**) must be the structure for isosilybin.



(**1**) R^1 = CH_2OH, R^2 = 3-OMe-4-OH-C_6H_3

(**2**) R^1 = 3-OMe-4-OH-C_6H_3, R^2 = CH_2OH



(**3**) R^1 = CH_2OH, R^2 = 3-OMe-4-OH-C_6H_3

(**4**) R^1 = 3-OMe-4-OH-C_6H_3, R^2 = CH_2OH

The problem of the absolute configuration of silybin at C-2′ and C-3′ has remained unsolved for some years. Whereas the absolute configuration of C-2 and C-3 of the flavanonol ring was established as 2*R*,3*R* with reasonable certainty by comparison of the c.d. spectrum of (**1**) (where the strong flavanonol chromophore overcomes the other one) with that of natural 2*R*,3*R*-flavanonols, the assignment of C-2′ and C-3′ of the benzodioxan ring was only tentative.[3] A recent publication[12] and our own results on the mild dehydrogenation of (**1**) show that 2,3-dehydrosilybin is optically inactive, in contrast with a preceding report,[3] racemization during the dehydrogenation being excluded by a deuterium exchange experiment.¶ This evidence indicates that natural silybin is a diastereoisomeric mixture of two compounds with the same configuration at C-2 and C-3, and opposite at C-2′ and C-3′ (therefore giving a racemic 2,3-dehydro derivative), even though it appears as a single compound on t.l.c. in many different solvents and also on h.p.l.c. (silica gel, MeCN–H_2O).** We have now found that if the ^1H n.m.r. spectrum of silybin is measured in benzene containing the

† *Present address:* Istituto di Biochimica Generale, Università di Milano, Italy.

‡ A more recent paper by Tittel and Wagner[8] reports that silybin shows two peaks in h.p.l.c., but surprisingly does not refer to isosilybin.

§ In the ^{13}C n.m.r. spectrum, the chemical shifts of corresponding carbons for (**1**) and (**2**) differ by 0·1 p.p.m., except for the benzodioxan bridgehead atoms (*ca.* 0·3 p.p.m.).

¶ The same happens for the related xanthonolignoid kielcorin.[12]

** The second peak for silybin observed by Tittel and Wagner[8] in h.p.l.c. of the crude extract of *Silybum marianum* may almost certainly be attributed to isosilybin.

minimum amount of pyridine to ensure solubility, the signals of 3'-H and of the methoxy group are split into two peaks of equal intensity with $\Delta\nu$ of 1—2 Hz (100 MHz). As the sample of silybin was completely free from the regio-isomer isosilybin, natural silybin must be a diastereoisomeric mixture of *ca.* 1 : 1 composition. A similar experiment with isosilybin in toluene and pyridine gave a similar doubling of the 3-H signal. Moreover the zero optical rotation of 2,3-dehydroisosilybin is consistent with isosilybin also being a diastereoisomeric mixture.

These results are understandable, after the recent results of synthetic work[9] and the isolation of isosilybin. They support the hypothesis of silybin being formed in the plant *via* a free radical oxidative coupling of dihydroquercetin and coniferyl alcohol,[6] which can give rise to a mixture of regio- and diastereo-isomers. Recently Schrall and Becker[13] have shown that silybin is indeed synthesized from these precursors by cell free extracts of *Silybum marianum*, and also by horseradish peroxidase *in vitro*.

We thank Inverni Della Beffa, Milano, for a generous gift of silybin and crude extracts, and Prof. P. Salvadori (Pisa) for c.d. spectra.

(*Received, 5th March* 1979; *Com.* 219.)

[1] H. Wagner, in 'Recent Flavonoid Research,' Akademiai Kiadò, Budapest, 1973, p. 51.
[2] G. Vogel, in 'New Natural Products and Plant Drugs with Pharmacological, Biological and Therapeutical Activity,' Springer, Berlin, 1977, p. 249.
[3] A. Pelter and R. Hänsel, *Chem. Ber.*, 1975, **108**, 790.
[4] R. Hänsel, J. Schulz, and A. Pelter, *Chem. Ber.*, 1975, **108**, 1482.
[5] H. Wagner, in 'Flavonoids and Bioflavonoids,' Elsevier, Amsterdam, 1977, p. 38.
[6] A. Pelter, quoted in R. Hänsel and H. Rimpler, *Deut. Apothek. Zeit.*, 1968, **108**, 1985.
[7] H. Wagner, P. Diesel, and M. Seitz, *Arzneim.-Forsch.*, 1974, **24**, 466.
[8] G. Tittel and H. Wagner, *J. Chromatography*, 1977, **135**, 499.
[9] L. Merlini, A. Zanarotti, A. Pelter, M. P. Rochefort, and R. Hänsel, preceding communication.
[10] A. R. Martin, S. K. Mallick, and J. F. Caputo, *J. Org. Chem.*, 1974, **39**, 1808. A few other 2,3-*cis*-benzodioxans, prepared in this laboratory, including 2-methyl-3-phenylbenzodioxan, show a $J_{2,3}$ value of 2 Hz.
[11] L. Merlini and A. Zanarotti, to be published.
[12] H. Nielsen and P. Arends, *Phytochemistry*, 1978, **17**, 2040.
[13] R. Schrall and H. Becker, *Planta Medica*, 1977, **32**, 27.