AMORITIN, AMORISIN, AND AMORILIN: THREE NEW PRENYLATED FLAVANONES FROM AMORPHA FRUTICOSA L.

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Abstract--- Three novel flavanones, amoritin, amorisin, and amorilin were isolated from the root bark of Amorpha fruticosa L. Their structures were determined on the basis of chemical and spectroscopic evidences.

The crude extracts and some flavonoid components of the fruit of Amorpha fruticosa L.(Leguminosae) show versatile biological activities: *insecticide*[1,2], *antifeedant*[3], *antiparasitic*[4], *antimicrobial*[5,6], hypotensive[7]. Concerning chemical or biological investigations of the other parts of the plant only sporadic data are available in the literature, so an investigation on the flavonoids of the leaves and root bark was started.

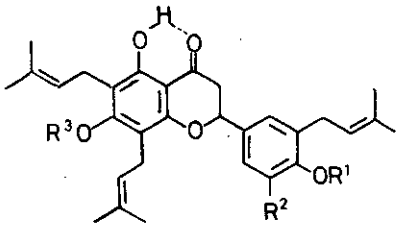
Three new oily flavanones resulted from chromatographic working up of the benzene soluble extract from the root bark: amoritin, $C_{31}H_{38}O_6$ (M^+ 506), amorisin, $C_{30}H_{36}O_6$ (M^+ 492), and amorilin, $C_{30}H_{36}O_5$ (M^+ 476). This communication reports the structures of amoritin, amorisin, and amorilin as 1, 2, and 3, respectively, based on spectroscopic evidences and chemical transformations.

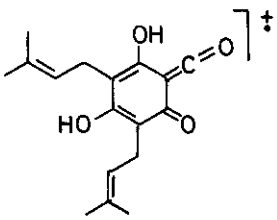
Amoritin gives positive ferric chloride test for phenolic hydroxyl group(s) in the molecule, in agreement with the strong OH-absorption at 3320 cm^{-1} in the IR spectrum. The UV spectrum of amoritin (λ_{max}^{MeOH} (lg ε): 298 (4.50) and 348 nm (4.05)) suggests[8] a flavanone type structure.

The ^{1}H-NMR spectrum showed signals at δ 1.73, 1.82 (15H and 3H, each br s, 6 x CH_3), 3.33 (6H, m, 3 x CH_2), and 5.23 (4H, m, 3 x CH + 2-H), for three C-linked 3,3-dimethylallyl (prenyl) side chains as well as signals at δ 12.61 and 6.35 for a chelated and non-chelated hydroxyl group. The meta coupling doublets at δ 6.91 and 6.75 (each 1H, J=2Hz) are characteristic for 2'- and 6'-protons[8] and indicate a 3',4',5'-trisubstituted ring B. In addition, a singlet at δ 3.81 indicates the presence of a methoxyl group, too; the 3-H's appear as a multiplet at δ 2.90. Methylation of amoritin with methyl iodide in acetone and in the presence of K_2CO_3 led to a mixture of a mono- and a dimethylated derivatives; the acetic anhydride-pyridine acetylation yielded only one diacetylated product. Both the dimethyl and diacetyl derivatives of amoritin show in their ^{1}H-NMR spectra the presence of a chelated hydroxyl group, indicating really three hydroxyl substituents for amoritin.

One of these is evidently in position 5, because it is the only one to chelatise. A second hydroxyl group is in position 7, as shown by the characteristic red-shift of the second band in the UV spectrum[9] of amoritin upon addition of NaOAc ($\lambda_{max}^{MeOH+NaOAc}$ (lg ε): 345 nm (4.73)).

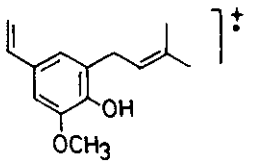
To locate the third hydroxyl, the methoxyl, and the three prenyl groups, the mass spectrum of amoritin is of diagnostic value.





$A^{\ddagger}$ (from 1 - 3)

1: $R^1=R^3=H$, $R^2=OCH_3$
2: $R^1=R^3=H$ $R^2=OH$
3: $R^1=R^2=R^3=H$
4: $R^1=H$, $R^2=OCH_3$, $R^3=CH_3$
5: $R^1=R^3=CH_3$, $R^2=OCH_3$
6: $R^1=R^3=COCH_3$, $R^2=OCH_3$



$B^{\ddagger}$ (from 1)

The well-known fragmentation pattern of flavanones[10,11] results in the formation of the ions $A^{\ddagger}$ and $B^{\ddagger}$ in the course of a retro-Diels-Alder reaction. Table 1 shows these ions, together with the more stable ions which arise by the loss of C_3H_7 and C_4H_7, as well as some other important/diagnostic fragments. The fragment m/e 288 shows clearly the substitution of ring A of amoritin: in addition to two hydroxyl groups, two prenyl groups are present, too. Since the positions 5 and 7 are hydroxy substituted, the prenyls are linked in positions 6 and 8. ^{13}C-NMR data[12] correspond with those of a 5,7-dihydroxy-6,8-diprenyl substituted ring A.

The fragment ion $B^{\ddagger}$ (m/e 218) from amoritin shows one hydroxyl, one methoxyl, and one prenyl substituent in ring B. Due to the magnetic non-equivalence of the protons 2' and 6' in 1 the prenyl rest is linked in 3' or 5' position. A comparison of the ^{1}H-NMR spectra of amoritin (1) and its diacetyl derivative (6) (the ^{1}H-NMR data of compounds 1-6 are in Table 2) shows the hydroxyl group in position 4' (meta to the 6' proton [13]) (Δ : +0.20). Thus the substitution pattern of ring B is of a 3'-prenyl-4'-hydroxy-5'-methoxy type, and the structure of amoritin corresponds to the formula 1[14].

The mono methylated derivative 4 has one methoxyl more than amoritin (1) (Table 1 and 2). The second methoxyl is at C-7, because the UV spectrum of 4 remained unaffected upon addition of NaOAc, and in the mass spectrum of 4, there is a greater parent fragment of 14 mass units (m/e 302) than in the spectrum of 1. In 5 all hydroxyl groups are methylated except the chelated hydroxyl (Table 1 and 2). Hence, structures 4 and 5 are assigned to the methylated derivatives of amoritin (1).

The diacetyl derivative of 1 can be formulated as 6, due to its ^{1}H-NMR and mass spectrometric data (δ 2.34 and 2.29 for two acetyl methyl group protons and 12.04 for the chelated hydroxyl; retro-Diels-

Table 1. Some diagnostic MS-fragments (% rel. int.) from flavanone derivatives 1 - 6.

Fragment	1	2	3	4	5	6
M^+	506(18)	492(5)	476(16)	520(12)	534(16)	590(60)
$[M-C_3H_7]^+$	463(2)	499(1)	433(2)	477(2)	491(3)	547(36)
$[M-C_4H_7]^+$	451(8)	437(2)	421(6)	465(4)	479(4)	535(16)
$[M-C_3H_7-C_4H_8]^+$	407(3)	393(2)	377(3)	421(2)	435(2)	491(100)
$[M-C_4H_7$-ring B$]^+$	260(6)	260(5)	260(12)	274(8)	274(14)	302(4)
A^+	288(4)	288(3)	288(4)	302(2)	302(6)	330(3)
$[A-C_3H_7]^+$	245(10)	245(8)	245(15)	259(11)	259(23)	287(12)
$[A-C_4H_7]^+$	233(16)	233(16)	233(32)	247(20)	247(25)	275(3)
$[A-C_3H_7-C_4H_8]^+$	189(38)	189(32)	189(71)	203(22)	203(32)	231(80)
B^+	218(7)	204(7)	188(10)	218(6)	232(10)	260(11)
$[B-C_3H_7]^+$	175(4)	161(4)	145(8)	175(6)	189(14)	217(34)
$[B-C_4H_7]^+$	163(5)	149(8)	133(30)	163(7)	177(11)	205(20)

Table 2. Chemical shifts (δ) of flavanone (1 - 6) protons in $CDCl_3$.

Comp.	5-OH (s)	2'-H (d)[a]	6'-H (d)[a]	2-H and CH (m) (4H)	CH_2 (m) (6H)	3-H (m) (2H)	CH_3 (s)	OCH_3 (s)	$COCH_3$ (s)
1	12.61	6.91	6.75	5.23	3.33	2.90	1.81(3H) 1.72(15H)	3.81	-
2	12.89	6.84	6.71	5.20	3.32	2.89	1.77(9H) 1.71(9H)	-	-
3[b]	12.32	7.17	7.17[c]	5.22	3.33	2.92	1.78(3H) 1.77(3H) 1.75(3H) 1.73(3H) 1.71(3H) 1.69(3H)	-	-
4	12.03	6.86	6.71	5.18	3.29	2.92	1.73(6H) 1.65(6H) 1.59(6H)	3.81 3.75	- -
5	12.03	6.86	6.80	5.27	3.35	2.98	1.73(9H) 1.64(9H)	3.86 3.82 3.75	-
6	12.04	7.06	6.95	5.16	3.11	2.93	1.73(6H) 1.65(6H) 1.56(6H)	3.79	2.34 2.29

[a] J=2 Hz; [b] 5'-H at δ 6.82, d, J=8.9 Hz; [c] dd, J=8.9 and 2 Hz.

Alder parent fragments at m/e 330 and 260).

The UV spectrum of amorisin (λ_{max}^{MeOH} (lg ε) : 298 (4.52) and 350 nm (3.99)) indicates a flavanone type structure[8]. ^{1}H-NMR and mass spectra (Table 1 and 2) show a very close relation to amoritin (1), except for a lesser molecular mass of 14 mass units. Methylation of amorisin led to a trimethylated product, which was identical to the dimethyl derivative 5 of amoritin. Thus the structure 2 is proposed for amorisin.

The UV spectrum of the third oily product, amorilin, shows also a flavanone structure[8] (λ_{max}^{MeOH} (lg ε) : 298 (4.50) and 352 nm (3.98)). The fragments m/e 260 and 288 in the mass spectrum indicate the same substitution of ring A (Table 1) as in amoritin (1) and amorisin (2). The signals at δ 6.82 (1H, d, J=8.9 Hz, 5'-H), 7.17 (1H, d, J=2.0 Hz, 2'-H) and 7.17 (1H, dd, J=8.9 and 2.0 Hz, 6'-H) in the ^{1}H-NMR spectrum (Table 2) of amorilin shows a 3',4'-disubstituted ring B with prenyl and hydroxyl groups. The chemical shifts of 2'-H and 6'-H also indicate a prenyl group in position 3' as well as a hydroxyl in position 4'[14]. Hence, structure 3 is proposed for amorilin.

References and Notes

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14. The alternative 2'-hydroxy-3'-methoxy-5'-prenyl substitution of ring B in amoritin (1) and 2'-hydroxy-4'-prenyl substitution of ring B in amorilin (3) were excluded on the basis of the carbon-13 chemical shifts of C-2 and C-1'. If ring B is 2'-hydroxy substituted these signals appear at about δ 75 and 125, respectively. By a non substituted, or 3'-mono-, 4'-mono-, and 3',4'-dioxygenated ring B the carbons 2 and 1' resonate at about δ 78-79 and 129-139, respectively. The signals at δ 78.5 and 133.2 in the spectrum of amoritin and at δ 78.7 and 133.8 in the spectrum of amorilin (note 12) indicated clearly a substitution pattern in ring B according to the formulas 1 and 3.

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