7. González, A. G., Barrera, J. B., Rozas, F. E., Hernández. C. Y., Espiñeira, H. and Nathan, P. J. (1983) *Phytochemistry* **22**, 2889.
8. Martínez, V. M., Sánchez, F. A. and Nathan, J. P. (1987) *Phytochemistry* **26**, 2577.
9. Bohlmann, F., Kramp, N., Gupta, K. R., King, M. R. and Robinson, H. (1981) *Phytochemistry* **20**, 2375.
10. González, A. G., Barrera, J. B., Hernández, C. Y., Rozas, F. E. and Domínguez, X. A. (1985) *Phytochemistry* **24**, 1847.

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8-PRENYLLUTEONE, A PRENYLATED ISOFLAVONE FROM *ERYTHRINA ERIOTRIOCHA*

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Key Word Index—*Erythrina eriotriocha*; Leguminosae; stem bark; 8-prenylluteone; 6,8-diprenylorobol; auriculasin; scandenone; cudraisoflavone A.

Abstract—The new isoflavone, 8-prenylluteone, has been isolated from the stem bark of *Erythrina eriotriocha* and its structure established by spectroscopic means and chemical transformations. The previously known prenylated isoflavones 6,8-diprenylorobol, auriculasin and scandenone have been also isolated. Cudraisoflavone-A has been shown to be identical with auriculasin.

INTRODUCTION

The genus *Erythrina* is widely known for its physiologically active alkaloids [1]. In recent years, however, there has been an increase in research efforts on the non-alkaloidal secondary metabolites, especially flavanoids and pterocarpans, of this genus [2–4]. As part of our investigation on Cameroonian medicinal plants in general and on the genus *Erythrina* in particular, we have continued [4] our study by investigating the constituents of *E. eriotriocha*. In this paper, we describe the isolation and structural determination of a new isoflavone (8-prenylluteone, **1**) along with three previously known isoflavones 6,8-diprenylorobol **4**, scandenone **5** and auriculasin **6**. The ^{13}C NMR data of **4**–**6** are reported for the first time. The structure of cudraisoflavone A **7** is incorrect, and is shown to be identical to auriculasin **6**.

RESULTS AND DISCUSSION

8-Prenylluteone (**1**), $C_{25}H_{26}O_6$ ([M^+] 422.1787, Calcd 422.1729), was isolated from the chloroform extract of the stem bark of *Erythrina eriotriocha* as described in the Experimental. The IR spectrum of (**1**) exhibited absorptions at 3415 (free OH), 3373 (chelated OH) and 1640 cm^{-1} (conj. carbonyl). The downfield signal in ^1H NMR at δ12.50 confirmed the presence of an intramolecular hydrogen bonded group at the C-5 position, while acetylation of **1** with acetic anhydride–pyridine yielded a tetraacetate (**2**), which did not respond to iron (III) test. Thus, **1** contains four hydroxyl groups (three free hydroxyls and one chelated hydroxyl). The signal in the ^1H NMR spectrum observed at δ7.98 is assigned to the C-2 proton of an isoflavone. This skeleton was supported by its UV spectrum (see Experimental) and by the following colour tests; positive to $FeCl_3$ (greenish-brown) and negative to Mg–HCl. The presence of two γ,γ-dimethylallyl (=prenyl) groups was shown in the ^1H NMR spectrum by four methyl signals (δ1.71, 1.74, 1.80 and 1.81), two 2H doublets (δ3.43 and 3.46; J = 7.1 Hz), Ar–CH_2–CH=C and two 1H triplets at δ5.18 and 5.22, J = 7.1 Hz, Ar–CH_2–CH=C). Furthermore, a typical ABX system at δ6.44 (*dd*, J = 7.2, 2.2 Hz) 6.52 (*d*, J = 2.2 Hz) and 6.98 (*d*, J = 7.2 Hz) showed the presence of three aromatic protons in B ring. The lack of further aromatic signals suggested that the H-6 and H-8 protons are absent [5]. In the EI mass spectrum, the molecular ion was detected at m/z 422 and other prominent fragments are shown in Fig. 1. The fragment ion peaks at m/z 288 and 134 caused by usual retro-Diels–Alder cleavage revealed information about the structure of (**1**). The ion m/z 288 resulted from the A ring and showed that this moiety possessed two prenyl groups at C-6 and C-8 in addition to two hydroxyls at C-5 and C-7. On the other

Part 12 in the series '*Erythrina* Studies'. For part 11 see ref. [4].
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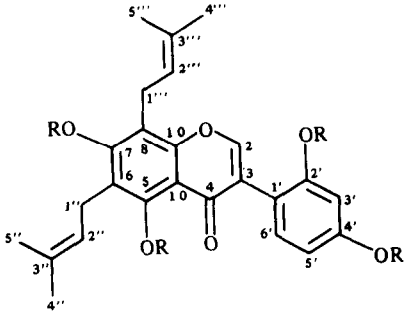

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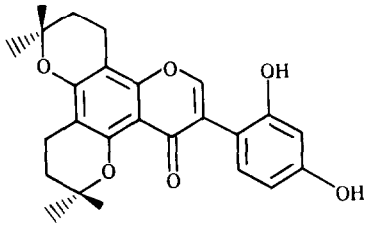
2 R = Ac



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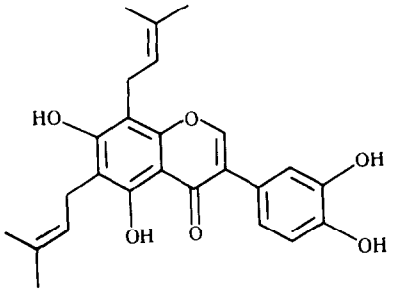

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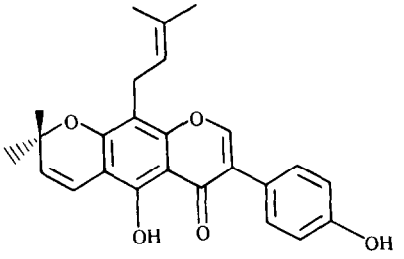

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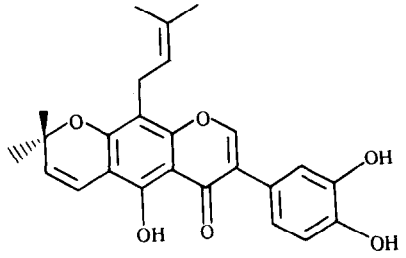

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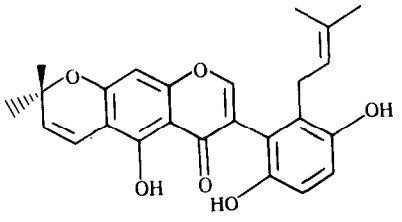

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hand, the ion *m/z* 134 arose from the ring B moiety and showed that this ring had two hydroxy groups whose relative positions had to be determined. On biogenetic grounds [6], it was assumed that there would be hydroxylation at C-4′. Furthermore, the absence of a bathochromic shift in the UV spectrum of **1** induced by a mixture of $AlCl_3$+HCl or NaOAc+H_3BO_3 [7] (see Experimental) is consistent with the formulation that the two hydroxyls in the B ring are not *ortho*. Support of the *meta* position of the two hydroxyls in ring B is given by the ^{13}C NMR spectrum which shows diagnostic signals at δ157.2 and 154.9 due to *meta* carbons bearing hydroxyls (see Table 1). Confirmation of this structural assignment was readily obtained by formic acid cyclization of (**1**) which afforded the *bis*-chromane derivative (**3**). Therefore, the structure of 8-prenylluteone was concluded to be 5,7,2′,4′-tetrahydroxy 6,8-di (γ,γ-dimethylallyl) isoflavone (**1**). The spectral data of 6,8-diprenylorobol **4**, scandenone **5**, and auriculasin **6** correspond to published data [8–10], and support the structures as indicated. The NMR data is shown in Table 1 for **4**–**6** with the ^{13}C NMR assignments given for the first time. However, the spectral data of **6** also closely matched that published for cudraisoflavone A **7** and its triacetate [11]. The reported mp of **6** was 176–178° [10] (EtOH) while **7** was 167–170° [11] ($CHCl_3$). The ^1H NMR are identical to those reported for **7** although we observed a significant small *meta* coupling of 1.4 Hz between 2′-H and 6′-H in the B ring of **6** (Table 1) that was not observed in **7** (probably due to lack of resolution). This secured the B ring substitution as shown in **6**. The assignment of 8-H in **7** at δ7.03 is also inconsistent with known compounds. The chemical shift range for 8-H is δ5.9–6.3 for similar isoflavones in $CDCl_3$. This information leads us to strongly suggest cudraisoflavone A **7** is identical to auriculasin **6**.

EXPERIMENTAL

Plant. material. *E. eriotriocha* stem bark was collected at Meiganga, in Adamaoua province of Cameroon, in June 1987. Voucher material documenting the collection was identified by

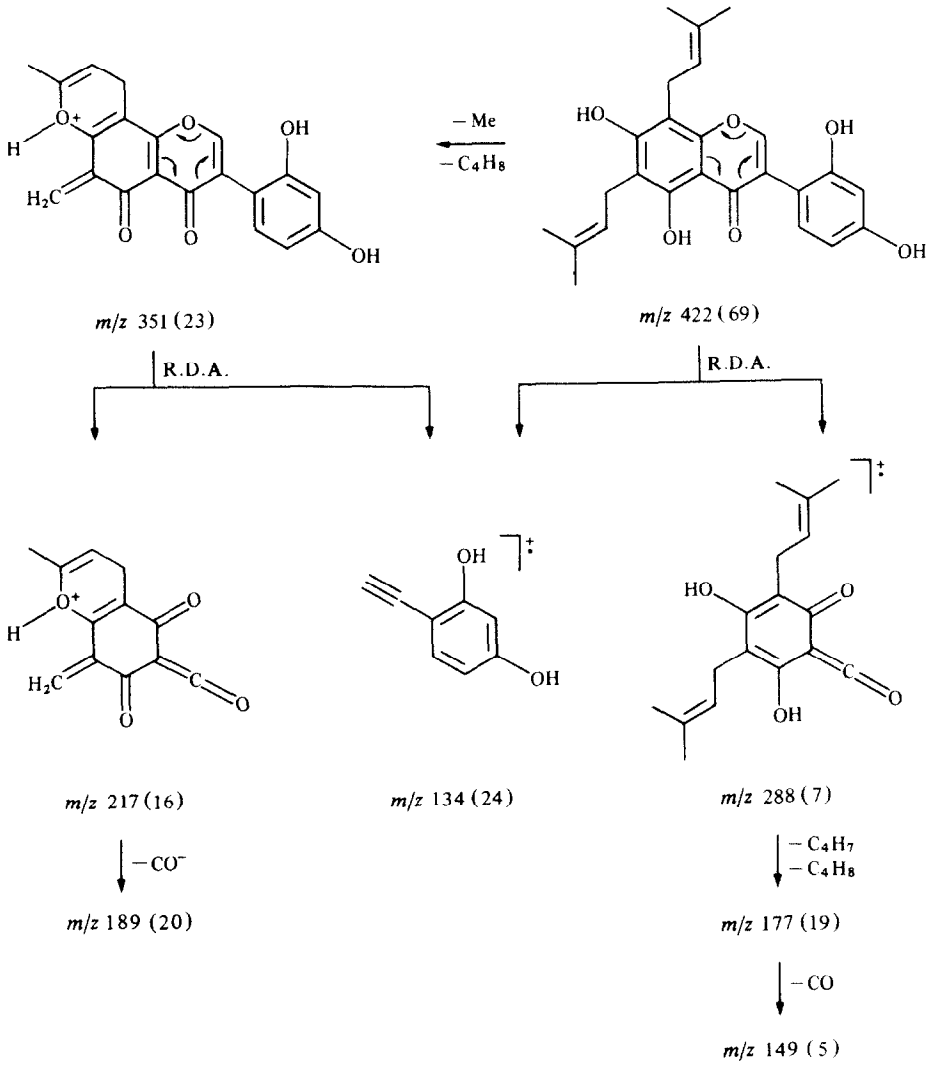

Fig. 1. Mass spectral fragmentations on 8-prenylluteone (1) showing prenyl substitution on the A ring.

the Director of the National Herbarium, Yaounde, Cameroon and is on deposit there.

Extraction and isolation. Dried ground stem bark (10 kg) was successively extracted in a Soxhlet with *n*-hexane, $CHCl_3$ and MeOH. Concentration under red. pres. gave respectively 60 g (0.6%) of hexane extract and 200 g (2%) of $CHCl_3$ extract. The MeOH extract consists mainly of tannins. Only the $CHCl_3$ extract was examined in this investigation. Part of this extract (100 g) was column chromatographed over silica gel (900 g) packed in *n*-hexanes. Gradient elution was effected with hexane–EtOAc, EtOAc and MeOH–EtOAc mixtures. A total of 200 fractions of *ca* 150 ml per fraction were collected and mixed on the basis of TLC. The pure compounds were obtained from the combined fractions after further purification by CC followed by prep. TLC.

The combined fractions 50–75 eluted with hexane–EtOAc (17:3) were concd to give a yellow sticky oil (3 g), which was subjected to CC on silica gel and eluted with *n*-hexane and increasing concentration of EtOAc in hexane, yielding three fractions A–C.

Fraction A, eluted with a mixture of hexane–EtOAc (10:1), slowly cryst. from petrol–EtOAc (19:1) to give 100 mg of scandenone (**5**) as yellow needles mp 168° (lit [15] 164–168°); UV λ_{max}^{MeOH} nm (log ε) 226 (4.56), 287 (4.72) and 338 (4.01); IR $\nu_{max}^{CDCl_3}$ cm^{-1} 3450, 3350, 1640, 1610, 1560 and 1620; ^1H NMR (see Table 1), ^{13}C NMR (see Table 1); EIMS *m/z* 404 (M^+) 389, 361, 349, 187 and 167.

Fraction B (300 mg) eluted with a mixture of hexane–EtOAc (10:2), was subjected to prep. TLC on silica gel with toluene–Me_2CO (10:3) and yielded auriculasin (**6**) which was cryst. from CH_2Cl_2 as yellow needles (60 mg) mp 178° (lit [10]mp 176–178°); UV λ_{max}^{MeOH} nm (log ε) 357 (3.54), 290 (4.67), 225 (4.45); + NaOMe: 357 (3%) 290 (4.57), 225 (4.62); + $AlCl_3$: 354 (3.73), 295 (4.57), 220 (4.50); $AlCl_3$ + HCl: 357 (3.50), 297 (4.48), 227 (4.42); + NaOAc: 357 (3.53), 290 (4.59); + NaOAC + H_3BO_3: 357 (3.51), 290 (4.44); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3150, 3280, 1640; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 420 (M^+ 43) 405 (100), 377 (15), 365 (11), 349 (3), 337 (5), 202 (14), 175 (4), 134 (12).

Fraction C (400 mg) was rechromatographed on a silica gel flash column eluted with hexane and increasing concn of EtOAc.

Table 1. ^{1}H NMR data and ^{13}C NMR assignments for 8-prenylluteone (**1**), 6,8-diprenylorobol (**4**), scandenone (**5**) and auriculasin (**6**) (TMS as int. standard)

	Compound							
	1 ($\delta CDCl_3$)		**4** (δacetone-d_6)		**5** ($\delta CDCl_3$)		**6** ($\delta CDCl_3$)	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	7.98 (1H, *s*)	160.5	8.25 (1H, *s*)	159.5	7.89 (1H, *s*)	152.8	7.90 (1H, *s*)	153.2
3	—	121.9	—	121.0	—	122.5	—	121.0
4	—	182.4	—	181.3	—	181.3	—	181.0
5	—	157.6	—	154.1	—	156.2	—	154.6
6	—	108.5	—	105.5	—	107.5	—	105.5
7	—	158.1	—	157.5	—	153.0	—	157.1
8	—	106.6	—	105.1	—	104.8	—	105.7
9	—	153.1	—	153.2	—	157.0	—	154.8
10	—	105.2	—	105.8	—	105.0	—	107.6
1′	—	112.5	—	125.7	—	123.5	—	123.4
2′	—	154.9	7.15(1H, *d*, $J = 2.0$ Hz)	110.1	7.60(1H, *d*, $J = 8.6$ Hz)	128.0	7.00(1H, *d*, $J = 1.4$ Hz)	128.0
3′	6.52(1H, *d*, $J = 2.7$ Hz)	105.6	—	144.9	6.57(1H, *d*, $J = 8.6$ Hz)	115.7	—	143.9
4′	—	157.2	—	145.5	—	154.7	—	144.6
5′	6.44(1H, *dd*, $J = 2.1$, 7.2 Hz)	111.2	6.87(1H, *dd*, $J = 2.0$, 8.1 Hz)	110.1	6.57(1H, *d*, $J = 8.6$ Hz)	115.7	6.85(1H, *d*, $J = 8.2$ Hz)	116.4
6′	6.98(1H, *d*, $J = 7.2$ Hz)	130.7	6.94(1H, *d*, $J = 8.1$ Hz)	121.5	7.60(1H, *d*, $J = 8.6$ Hz)	128.0	6.83(1H, *dd*, $J = 1.4$ and 8.2 Hz)	122.6
1″	3.46(2H, *d*, $J = 7.1$ Hz)[a]	21.7	3.51(2H, *d*, $J = 7.0$ Hz)[a]	21.6	3.41(1H, *d*, $J = 7.0$ Hz)	21.2	3.40(2H, *d*, $J = 7.2$ Hz)	21.3
2″	5.22(1H, t, $J = 7.1$ Hz)[b]	121.2	5.24(1H, *t*, $J = 7$ Hz)[b]	123.0	5.18(1H, *t*, $J = 7.0$ Hz)	122.1	5.17(1H, *t*, 7.2 Hz)	121.8
3″	—	135.8		134.9	—	131.4	—	131.7
4″	1.82(3H, *s*)[c]	25.8	1.98(3H, *s*)[c]	25.6	1.81(3H, *s*)	25.7	1.81(3H, *s*)	25.7
5″	1.74(3H, *s*)[d]	17.9	1.81(3H, *s*)[d]	17.7	1.69(3H, *s*)	17.8	1.68(3H, *s*)	17.9
6″	—	—	—	—	—	—	—	—
1‴	3.43(2H, *d*, $J = 7.2$ Hz)	21.7	3.48(2H, *d*, $J = 7.1$ Hz)[a]	21.6	—	—	—	—
2‴	5.18(1H, *t*, $J = 7.2$ Hz)	120.9	5.19(1H, *t*, $J = 7.1$ Hz)[b]	122.2	—	77.7	—	77.9
3‴	—	134.5	—	134.0	5.63 (1H, *d*, $J = 10$ Hz)	130.2	5.65 (1H, *d*, $J = 10$ Hz)	128.0
4‴	1.80(3H, *s*)[c]	25.9	1.96(3H, *s*)[c]	25.6	6.74(1H, *d*, $J = 10$ Hz)	121.9	6.73(1H, *d*, $J = 10$ Hz)	115.9
5‴	1.71(3H, *s*)[a]	17.9	1.78(3H, *S*)[d]	17.7	1.47(3H, *s*)	28.2	1.43(3H, *s*)	28.1
6‴	—	—	—	—	1.47(3H, *s*)	28.2	1.43(3H, *s*)	28.1
5-OH	12.50(1H, *b*)		13.65(1H, *s*)					
2′-OH								
3′-OH			8.40(1H, *br*)					
4′-OH			8.10(1H, *br*)					

[a–d] Assignments may be reversed within column.

100 fractions of 25 ml were collected and combined on the basis of TLC. Fractions 75-100, eluted with a mixture of hexane-EtOAc (5: 2) were subjected to prep. reverse phase TLC with MeOH-H20 (10:3) to yield 6,8-diprenylorobol (4) as colourless crystals (30mg) from CH2Cl2-hexane and 8-prenylluteone (1) as an oil (35 mg).

6,8-Diprenylorobol (4). Ry: 0.45 (toluene-M%CO 10:3) mp 156° (lit. 155-156°) [14], UV 2 ~oH nm (log r,): 348 (3.36), 270 (4.43); +NaOMe: 352 (4.07), 283 (4.27); +AICI3:338 (3.36), 293 (4.15), 274 (4.38); + A1CI3+ HCI: 338 (3.10), 274 (4.37); + NaOAc: 347 (3.43), 272 (4.37);+NaOAc+H3BO3:343 (3.36), 290sh (4.30), 271 (4.36); IR v c~cl3 cm-l: 3480, 3250, 1640, 1622, 1215, 728; IH NMR (see Table 1), 13C NMR (see Table 1); EIMS *m/z* 422 (M + 69), 405 (11), 379 (47), 367 (39), 351 (23), 323 (29), 311 (53), 288 (6), 217 (15), 134 (25); IH and 13CNMR (see Table 1).

8-Prenylluteone (1). Oil, Rs 0.48 (toluene-Me2CO 10: 3); UV 2 ~:oH nm (log e) 325 (3.76), 291sh, (4.76), 270 (4.26); +NaOMe: 337 (4.21), 287 (4.32); + AICI3:357 (3.04), 303sh, (4.04), 282 (4.30); + AICI3+ HCI: 357 (3.04), 303sh, (4.04), 282 (4.30); + NaOAC: 336 (4.23), 285 (4.34); +NaOAc+H3BO3:315 (3.77), 287sh, (4.75), 275 (4.26); HRMS *m/z* 422.1787 (calcd for CH25H2606 422.1729), EIMS *m/z* (rel. int.) 422 (M + 69), 405 (11), 379 (47), 367 (39), 351 (23}, 323 (29), 311 (53), 288 (7), 217 (16), 189 (20), 177 (l9), 134 (25), 69 (49), 56 (50), 43 (45), 41 (100).

Cyclization of 8-prenylluteone (1). A soln of 8-prenylluteone (5 mg) in HCO2H (2 ml) was refluxed for 1 hr. The soln was poured into cold H20 and extracted with CHCI 3. The extract was washed successively with aq. NaHCO 3and HzO, dried with Na2SO,, and evapd to dryness. Prep TLC of residue on silica gel (toluene-Me2CO 5:1) yielded the bis (dihydropyran) derivative (3) (3.5 mg, 70%), mp 106°; IR vC~3 3350, 1640, 1583, 1423, 1205, 1131; 1HNMR: 61.39 (6H, s, 2 × Me chromane ring), 1.40 (6H, s, 2 × Me chromane), 1.85 (2H, t, J = 6.8 Hz, methylene proton next to sp3-C, Ar-CH2CH2), 1.87 (2H, *t*, J = 6.8 Hz) methylene proton next to sp3 Ar-CH2CH~) 2.64 (2H, t, J = 6.9 Hz, methylene proton of chromane, next to aromatic ring), 2.81 (2H, t, 6.9 Hz, methylene proton of chromane next to aromatic ring), 4.84 (1H, s, exchangeable to D20, 4'-OH), 6.41 (1H, *dd*, J = 8.1 and 2.0 Hz, H-5'), 6.53 (1H, d, *J* = 2 Hz, H-Y), 6.93 (1H, d, J=8.1 Hz, H-6'), 7.83 (1H, s, H-2), 9.84 (IH, *brs*, exchangeable with DzO, 2'-OH).

Acetylation of 8-prenylluteone (1). Acetylation of 8-prenylluteone (6 mg) with AczO (2 ml) in pyridine (3 ml) followed by the customary work-up afforded 8-prenylluteone tetraacetate (2) as an oil (5 mg) which gave a negative response to the FeCI3 test: v c~13 cm-~ 1765, 1640, 1610, 1420, 1360, 1180, 1120; ~H NMR 61.64 (3H, s, *cis* Me, prenyl), 1.71 (3H, s, Me, prenyl), 1.75 (3H, s, Me, prenyl), 1.78 (3H, s, Me, prenyl), 2.20 (3H, s, Ac), 2.30 (3H, s, Ac), 2.35 (3H, s, Ac), 2.37 (3H, s, Ac), 3.38 (2H, d, J = 7 Hz, methylene protons), 3.42 (2H, d, J = 7 Hz, methylene protons), 5.08 (1H, t, J = 7 Hz, vinyl proton), 5.13 (IH, t, J =7 Hz, vinyl proton), 7.03 (1H, *dd*, J = 7.8 and 2 Hz, H-5'), 7.06 tlH, d, J =2 Hz, H-3'), 7.28 (IH, *d*, J = 7.8 Hz. H-6'), 7.85 (1H, s, H-2~.

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REFERENCES

1. Dyke, S. F. and Quessy, D. 0981) in *The Alkaloids, Chemistry and Physiolo.qy* (Rodrigo, R. G. A., ed.) Vol. 18, p. I. Academic Press, New York.
2. Kamat, V. S., Chuo, F. Y., Kubo, I. and Nakanishi, K. (1981) *lteterocycles* 15, 1163.
3. Mitscher, L. A., Gollapudi, S. R., Gerlach, D. C., Drake, S. D., Veliz, E. A. and Ward. A. J. {1988) *Phytochemistry* **27,** 381.
4. Rattanaporn, P., Tempcsta, M. S., Fomum, Z. T., and Mbafor, J. T. (1988) *J. Nat. Prod.* 51, 611.
5. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, Berlin.
6. Ebel, E. and Hahlbrock, K. (1982) in *The Flavonoids Advances in Research* (Harborne, J. B. and Mabry, T. J. eds), p. 649. Chapman & Hall, New York.
7. Markham, K. R. (1982) in *7~,chniques~Flavonoid Identification,* p. 43. Academic Press, New York.
8. Askok, K. S., Ram, P., Gopalakrishna, T., Werner, lq. and Serengolan, V. G. (1980) *Phytochemistry* 19, 929.
9. Peller, A. and Stainton, P. (1966) *J. Chem. Soc.* 701.
10. Minhaj, N., Khan, H., Kapoor, S. R and Zaman, A. (1976) *Tetrahedron* 32, 749.
11. Sun, N. J., Chang, C. J. and Cassady, J. M. (1988) *Phytoehemistry* 2% 951.