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CASSIADININE, A CHROMONE ALKALOID AND (+)-6-HYDROXYMELLEIN, A DIHYDROISOCOUMARIN FROM *CASSIA SIAMEA*

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Key Word Index—*Cassia siamea*; Leguminosae; alkaloid; cassiadinine; dihydroisocoumarin; (+)-6-hydroxymellein; 5-acetonyl-7-hydroxy-2-methylchromone; cycloart-23-ene-3β,25-diol; betulin; friedelin; γ-sitosterol.

Abstract—Cassiadinine, a new chromone alkaloid and (+)-6-hydroxymellein, a new dihydroisocoumarin isomer have been isolated together with three triterpenoids, cycloart-23-en-3β,25-diol, friedelin and betulin, a chromone derivative, 5-acetonyl-7-hydroxy-2-methylchromone and γ-sitosterol from the petrol and chloroform extracts of the flowers of *Cassia siamea*.

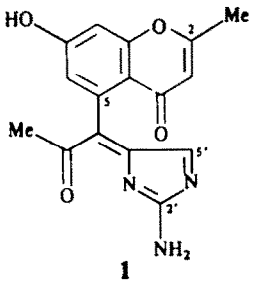
INTRODUCTION

Cassia siamea Lam. (Leguminosae) is one of the commonest trees of India. All its parts have been extensively studied and are known to yield anthraquinones [1–3], anthrones [1], flavones [4, 5], a chromone [5–7], a dioxaphenalene and its hydrochloride [4, 7], triterpenoids [2, 8, 9], alkaloids [7, 9, 10], a stilbene [11] and *p*-coumaric acid [5]. The sterol constituents [12] of its flowers were also studied. Our reinvestigation of the flowers has resulted in the isolation of a new chromone alkaloid, cassiadinine (**1**) and a new isomeric form of the dihydroisocoumarin, (+)-6-hydroxymellein (**2**) along with a number of known triterpenoids and a chromone. The present paper describes the isolation and elucidation of the structures of **1** and **2**.

RESULTS AND DISCUSSION

The basic fraction of the chloroform extract of the flowers of *C. siamea* afforded **1**, while the petrol extract and the non-basic fraction of the extract furnished **2** in addition to the known compounds, 5-acetonyl-7-hydroxy-2-methylchromone (**4**), cycloart-23-ene-3β,25-diol (**7**), friedelin, betulin and γ-sitosterol.

The alkaloid fraction of the chloroform extract of the flowers of *C. siamea* was separated and taken up in



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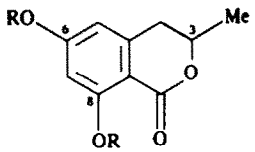

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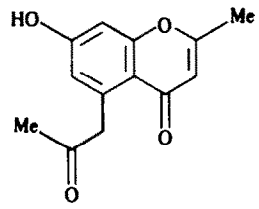

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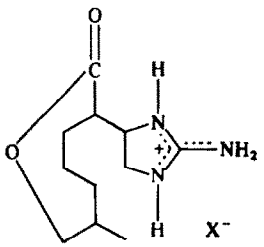

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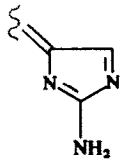

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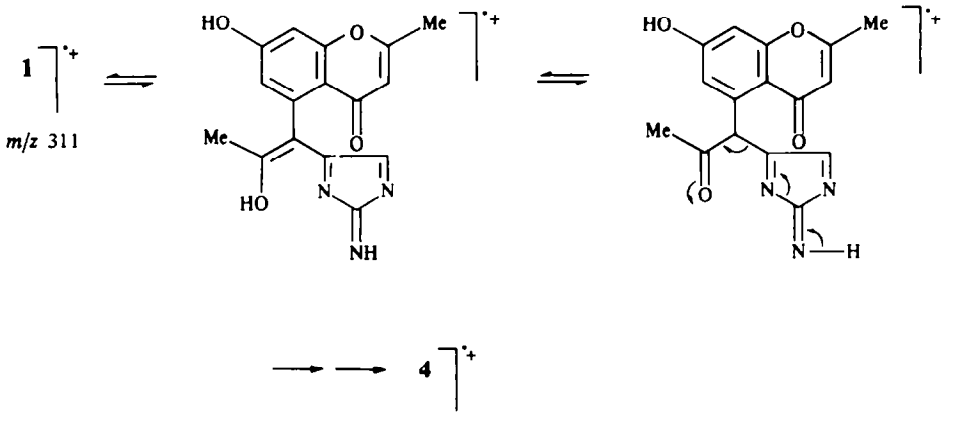
dichloromethane to give a greenish yellow fluorescent solution containing a single compound (TLC). From it only a small fraction of **1** could be obtained in crystalline form, mp 80° (dec.) (dichloromethane–petrol); all other attempts at crystallization from this or other solvent systems failed. Compound **1** was very unstable and its dichloromethane solution on concentration deposited a deep blue amorphous solid consisting of a mixture of **1** and a more polar compound (TLC) which could not be separated. An amorphous variety of **1** (containing traces of the blue compound) obtained by adding petrol to the concentrated dichloromethane solution was used for its reactions. It gave a reddish brown colour with ferric chloride. But, it neither furnished any pure product on treatment either with acetic anhydride or diazomethane, nor could the starting material be recovered from the reactions. Valuable information on the structure of **1** was obtained from its acid hydrolysis and comparison of its UV, IR, ^{1}H NMR and mass spectra with those of **4**, co-occurring with **1**. Acid hydrolysis of **1** afforded **4**. The UV spectrum of **1** exhibited absorption maxima (ethanol) at 243, 251 and 390 nm. The first two values are similar to those of **4** and the last one indicated a highly conjugated structure. The UV maxima of **1** in the presence of added alkali compared favourably with those of **4**. A broad IR band at 3420–2980 cm^{-1} indicated the presence of hydroxyl and/or NH in **1**. The presence of a chromone carbonyl and another conjugated carbonyl group was also evident from the appearance of bands at 1670 and 1695 cm^{-1}, respectively. Its ^{1}H NMR signals at δ2.21 (3H, *s*), 2.34 (3H, *s*), 5.96 (1H, *s*), 6.27 (1H, *d*, J = 1.5 Hz) and 6.38 (1H, *d*, J = 1.5 Hz) tallied well with those due to acetyl, 2-Me, H-3, H-8 and H-6 of **4**, respectively. However, the signal due to 5-CH_2–CO of **4** was absent from the spectrum of **1**; instead two additional signals at δ1.81 (2H, *s*, exchangeable with D_2O) and 6.23 (1H, *s*) were found. Compound **1** is, therefore, assumed to be constructed by attachment of a nitrogenous moiety to the α-carbon of the C-5 side chain of **4**. This attachment involved a carbon–carbon double bond, since acid hydrolysis of **1** afforded **4** and its IR spectrum showed a conjugated carbonyl absorption band at 1695 cm^{-1}. The mass spectrum of **1** exhibited $[M]^+$ at *m/z* 311 and another ion at *m/z* 283 $[M-CO]^+$ in addition to all the ion peaks observed in the spectrum of **4**. Thus, the nitrogenous moiety in **1** giving the ^{1}H NMR signals for three protons [at δ1.81 (2H) and 6.23 (1H)] has 81 mass units. Of the two possible compositions, $C_3H_3N_3$ and C_4H_3NO, for this moiety, both possessing three protons, the former was favoured, as any structure with the latter is neither feasible from the biogenetic point of view, nor could explain the observed ^{1}H NMR spectral features. The occurrence of the alkaloid, chaksine (**5**) in *Cassia absus* [13], a plant of the same genus suggested the presence of a guanidine moiety in **1** and its $C_3H_3N_3$ component probably possessed the partial structure **6**. Thus, cassiadinine is assigned the structure **1**; however, the stereochemistry of the double bond linking the chromone and the nitrogenous moieties remains unsettled. The $[M]^+$ of **4** possibly arises from that of **1** as shown in Scheme 1. To our knowledge, the previously reported naturally occurring chromone alkaloids are only five in number [14, 15]. Isolation of **1** makes an addition to this class.

Compound **1** is possibly formed from **4**; the latter may first undergo attachment with a two carbon unit and then with a guanidine moiety. The two carbon unit may arise through C-prenylation of the carbon which is both benzylic and α to a carbonyl, followed by oxidative cleavage of the prenyl group. The occurrence of terpenoids in the flowers of *C. siamea* tends to support this contention, but we have no supporting experimental evidence.

A light yellow amorphous solid (CS-1), mp 210–212°, $[\alpha]_D^{25}$ + 18.9° ($CHCl_3$), isolated from the chloroform extract of the flowers gave two very close spots in TLC, of which the upper one was due to a yellow compound. Their attempted separation by fractional crystallization or chromatography failed. The component of CS-1 giving the lower TLC spot was separated as its diacetate by column chromatography. The diacetate, mp 110°, $[M]^+$ 278, $[\alpha]_D^{25}$ + 138.7° ($CHCl_3$; *c* 0.053), exhibited UV and IR (1662 cm^{-1}, dihydroisocoumarin carbonyl) spectra similar to those of dihydroisocoumarin derivatives [16]. Its ^{1}H NMR spectrum indicated it to be either 6,8-diacetoxy-3-methyl-3,4-dihydroisocoumarin (**3**) or 5,7-diacetoxy-2-methylchromanone (**9**). However, its mass spectral fragmentation pattern favoured structure **3**. Hydrolysis of the diacetate with 0.5 N methanolic HCl afforded (+)-6-hydroxymellein (≡ 6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin, **2**), mp 211° (lit. [16]



Scheme 1.

211°); $[\alpha]_D^{25}$ +44.20 (EtOH; *c* 0.04) (lit. [16] −63° and [17] −54° for the (−)-variety). Finally, the identity of **2** was established by comparing it and its diacetate (**3**) with 5,7-dihydroxy-2-methylchromanone (**8**) and **9** respectively. Compound **8** was prepared [18] from phloroglucinol and crotonic anhydride.

(−)-6-Hydroxymellein was first isolated from a mutant of *Aspergillus terreus* [16] and then from *Daucus carota* [17] roots stored under conditions of stress and the culture broth of *Pyricularia oryzae* [19]. The racemic mixture was synthesized by Henderson and Hill [20]. To our knowledge, this is the first isolation of (+)-6-hydroxymellein (**2**) from a natural source and the first occurrence of a dihydroisocoumarin in the genus *Cassia*.

The other constituents were identified either by direct comparison with authentic samples or by comparison of their reported physical (mp and $[\alpha]_D$) and spectral (UV, IR, ^{1}H NMR and MS) data.

EXPERIMENTAL

Mps: uncorr.; UV: 95% EtOH; ^{1}H NMR: δ values in ppm downfield from TMSi; MS: 70 eV; chromatography on silica gel (100–200 mesh) unless otherwise stated; TLC spots visualized in UV light and on exposure to I_2 vapour; homogeneity of compounds established by TLC and MS.

Extraction. Dried and powdered flowers (600 g) of *C. siamea* collected from Midnapore District, West Bengal, India during October were extracted exhaustively in a Soxhlet apparatus with petrol (60–80°) and then with $CHCl_3$. The residue obtained by concn of the petrol extract was found to be devoid of any alkaloid, while that of the $CHCl_3$ extract gave a positive test with Dragendorff's reagent indicating the presence of alkaloid. The basic fraction of the $CHCl_3$ extract was separated by extraction with 5% citric acid soln. The concentrate of the petrol extract and non-basic fraction of the $CHCl_3$ extract were separately chromatographed over silica gel (60–120 mesh) using solvents and solvent mixtures of increasing polarity.

Compounds isolated from petrol extract. Friedelin. The light yellow waxy solid obtained from the petrol–C_6H_6 (1:1) eluate fractions showing one major spot in TLC, was chromatographed twice and then crystallized from $CHCl_3$–petrol to afford friedelin as colourless needles (5 mg), mp 254°, $[\alpha]_D^{25}$ −11.9° ($CHCl_3$; *c* 0.10) identical with an authentic sample in all respects (mmp, co-TLC and IR).

γ-Sitosterol. The residue obtained from the C_6H_6–$CHCl_3$ (1:1) eluates furnished γ-sitosterol after rechromatography followed by crystallization from petrol as flakes (30 mg), mp 148–150°, $[\alpha]_D^{25}$ −35° ($CHCl_3$; *c* 0.1); acetate, mp 140°.

Cycloart-23-en-3β,25-diol (**7**). The residue obtained from the earlier $CHCl_3$–MeOH (19:1) eluate fractions was chromatographed twice. The concentrate of the $CHCl_3$ eluate fractions afforded **7**, crystallizing from $CHCl_3$–petrol as colourless needles (5 mg), mp 198–199° (lit. [21] 198–199°), $[\alpha]_D^{25}$ +41.6° ($CHCl_3$; *c* 0.09); acetate (Ac_2O–pyridine), mp 150°. The identity of **7** was established from its spectral features and finally by direct comparison with an authentic sample [21].

Betulin. The dark brown residue obtained from the later $CHCl_3$–MeOH (19:1) eluates was chromatographed twice when betulin was obtained as a colourless amorphous solid (3 mg), mp 250–252°, $[\alpha]_D^{25}$ +20.2° ($CHCl_3$; *c* 0.08), identical in all respects with an authentic sample.

Separation of the alkaloid cassiadinine (**1**). The concentrate of the $CHCl_3$ extract was repeatedly extracted with 5% citric acid soln till the fresh extract showed negative test with Dragendorff's reagent. The combined acid extract was cooled, basified with $NaHCO_3$ and extracted with CH_2Cl_2. Crystalline cassiadinine (**1**) could be obtained only once from a small fraction of the CH_2Cl_2 soln by careful concn and dilution with petrol as green needles (3 mg), mp 80° (dec.); UV λ_{max}^{EtOH} nm: 243, 251 and 390; $\lambda_{max}^{EtOH+HCl}$ nm: 246.5, 305 and 330.5; $\lambda_{max}^{EtOH+NaOH}$ nm: 254, 279 and 334; IR ν_{max}^{KBr} cm^{-1}: 3420–2980 (OH and NH), 1695 (conjugated C=O), 1670 (chromone C=O), 1620, 1540, 1480, 1280, 1170 and 870; ^{1}H NMR (80 MHz, $CDCl_3$): δ1.81 (2H, *s*, exchangeable with D_2O, 2′-NH_2), 2.21 (3H, *s*, Ac), 2.34 (3H, *s*, 2-Me), 5.96 (1H, *s*, H-3), 6.23 (1H, *s*, H-5′), 6.27 (1H, *d*, *J* = 1.5 Hz, H-8) and 6.38 (1H, *d*, *J* = 1.5 Hz, H-6); ^{1}H NMR (200 MHz, $CDCl_3$): δ1.80 (*hump*, exchangeable with D_2O, 2′-NH_2), 2.25 (3H, *s*, Ac), 2.42 (3H, *s*, 2-Me), 6.15 (1H, *s*, H-3), 6.38 (1H, *s*, H-5′), 6.58 (1H, *d*, *J* = 1.5 Hz, H-8), 6.68 (1H, *d*, *J* = 1.5 Hz, H-6). EIMS *m/z* (rel. int.): 311 $[M]^+$ (27.8), 283 $[M-CO]^+$ (21.6), 232 $[M-C_3H_3N_3]^+$ (17.3), 190 (53.7), 162 (18.5), 161 (20.4), 150 (24.1), 122 (15.0), 121 (24.1) and 43 (100). All attempts to crystallize **1** present in the remaining CH_2Cl_2 soln by using CH_2Cl_2–petrol or any other solvent systems failed. It was almost completely adsorbed on neutral alumina during attempted purification by CC.

Compounds isolated from the non-basic part of the $CHCl_3$ *extract. CS-1* [≡ *mixture of* (+)-*6-hydroxymellein* (**2**) *and another uncharacterized compound*]. The light brown residue obtained by concn of the early C_6H_6 eluate fractions on repeated chromatography [eluent in the final stage: $CHCl_3$–MeOH (19:1)] followed by crystallization from $CHCl_3$–petrol afforded CS-1 as a light yellow solid (12 mg), mp 210–212°; $[\alpha]_D^{25}$ +18.9° ($CHCl_3$; *c* 0.074); IR ν_{max}^{KBr} cm^{-1}: 3250, 1690, 1650, 1580, 1530, 1490, 1390, 1260, 1180, 1120, 1070 and 730. It showed two almost overlapping spots in TLC; attempts to resolve this mixture to its components by repeated fractional crystallizations, and careful column and prep. TLC met with failure.

5-Acetonyl-7-hydroxy-2-methylchromone (**4**). The residue obtained from the early $CHCl_3$–MeOH (19:1) eluates on rechromatography followed by crystallization from $CHCl_3$–petrol furnished **4** as colourless needles (20 mg), mp 210° (lit. [6] 210°); UV λ_{max}^{EtOH} nm (log *ε*): 243 (4.13), 251 (4.16) and 293 (3.97); $\lambda_{max}^{EtOH+NaOH}$ nm (log *ε*): 257 (4.30) and 333.5 (4.04); IR ν_{max}^{KBr} cm^{-1}: 3200–2500 (OH), 1730 (Ac), 1660 (chromone C=O), 1590, 1520, 1460, 1410, 1340, 1280, 1160, 1110, 1050, 1010, 940 and 840; ^{1}H NMR (80 MHz, $CDCl_3$): δ2.29 (3H, *s*, Ac), 2.40 (3H, *s*, 2-Me), 4.20 (2H, *s*, 5-CH_2–), 5.95 (1H, *s*, H-3), 6.45 (1H, *d*, *J* = 1.5 Hz, H-8) and 6.60 (1H, *d*, *J* = 1.5 Hz, H-6); EIMS *m/z* (rel. int.): 232 $[M]^+$ (17.9), 190 (100), 162 (11.9), 161 (13.6), 150 (4.6), 122 (3.9), 121 (2.3) and 43 (23.3). On acetylation (Ac_2O–pyridine, room temp., 24 hr) **4** furnished its monoacetate, mp 131° (lit. [6] 132°).

Hydrolysis of cassiadinine (**1**). Compound **1** (10 mg) was refluxed with 5 ml of 0.5 N ethanolic HCl for 8 hr. Usual work up of the reaction mixture followed by chromatography of the product furnished **4** (eluate: $CHCl_3$–MeOH, 19:1), crystallizing from $CHCl_3$–petrol as almost colourless needles (2 mg), mp 210°. The identity of **4** was confirmed by direct comparison with a sample isolated from this plant.

(+)-*6-Hydroxymellein diacetate* (**3**). CS-1 (8 mg) was acetylated (Ac_2O–pyridine, 24 hr, room temp.) and the product chromatographed using $CHCl_3$–MeOH (99:1) as eluent to give a colourless solid which exhibited a major spot in TLC. Its rechromatography furnished **3**, crystallizing from $CHCl_3$–petrol as colourless needles (3.5 mg), mp 110°; $[\alpha]_D^{25}$ +138.7° ($CHCl_3$; *c* 0.053); UV λ_{max}^{EtOH} nm: 244 and 289; IR ν_{max}^{KBr} cm^{-1}: 2920, 2850, 1775 and 1200 (phenolic acetate), 1662 (dihydroisocoumarin C=O), 1615, 1570, 1460, 1370, 1252, 1132, 1060, 1020 and 795; ^{1}H NMR (80 MHz, $CDCl_3$): δ1.45 (3H, *d*, *J* = 6.5 Hz, 3-Me), 2.28 and 2.33 (each 3H, *s*, 2 OAc), 2.90 (2H, *d*, *J* = 7 Hz, H_2-4),

4.61 (1H, *sextet*, $J = 7$ Hz, H-3), 6.85 and 6.95 (each 1H, *d*, $J = 2$ Hz, H-5 and H-7); EIMS *m/z* (rel. int.): 278 $[M]^+$ (9.7), 263 $[M-Me]^+$ (10.9), 236 $[M-CH_2CO]^+$ (100), 221 $[M-CH_2-CO-Me]^+$ (42.7), 194 $[M-2CH_2CO]^+$ (100), 179 $[M-2CH_2CO-Me]^+$ (97.4), 176 $[M-2CH_2CO-H_2O]^+$ (98.8), 165 (97.6), m*: 159.67 (194 → 176), 150 (98.8), 122 (98.7) and m*: 116 (194 → 150).

Hydrolysis of (+)-6-hydroxymellein diacetate (3): *isolation of (+)-6-hydroxymellein* (2). A soln of the diacetate 3 (2.5 mg) in 2 ml of 0.5 N methanolic HCl was kept at room temp. for 48 hr and then worked up in the usual way to yield a product whose spot corresponded to the lower TLC spot of CS-1. On crystallization from Me_2CO–petrol it furnished (+)-6-hydroxymellein (2) as almost colourless needles (1 mg), mp 211° (lit. [16] 211°); $[\alpha]_D^{25}$ +44.2° (EtOH; *c* 0.04) (lit. [16] −63° and [17] −54° for the (−)-variety); UV λ_{max}^{EtOH} nm: 267 and 303 (lit. [17] 269 and 305); IR ν_{max}^{KBr} cm^{-1}: 3380–3200 (OH), 2920, 2850, 1660 (dihydroisocoumarin C=O), 1630, 1585, 1525, 1460, 1380, 1260, 1170, 1120, 1067, 1025 and 795 (lit. [17] 3420, 3220, 1664, 1635 and 1598).

5,7-Dihydroxy-2-methylchromanone (8). It was prepared from phloroglucinol and crotonic anhydride in the presence of dry $AlCl_3$ following the literature procedure [18] and obtained as light yellow needles, mp 175° ($CHCl_3$–petrol); UV λ_{max}^{EtOH} nm: 289 and 322; IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1675 (chromanone C=O), 1540, 1525, 1490, 1370, 1325 and 1185. Compound 8 (5 mg) on acetylation (Ac_2O–pyridine, 24 hr, room temp.) furnished 9 (3 mg), mp 98° ($CHCl_3$–petrol); UV λ_{max}^{EtOH} nm: 258 and 313; IR ν_{max}^{KBr} cm^{-1}: 1770 and 1220 (phenolic acetate), 1685 (chromanone C=O), 1620, 1570, 1440, 1370, 1262, 1190, 1180, 1135, 1078, 1030 and 895; ^1H NMR (80 MHz, $CDCl_3$): δ 1.48 (3H, *d*, $J = 6.6$ Hz, 2-Me), 2.28 and 2.35 (each 3H, *s*, 2 OAc), 2.59 and 2.60 (each 1H, *d*, $J = 6.6$ and 8.8 Hz, H_2-3), 4.46–4.90 (1H, *m*, H-2), 6.46 (1H, *d*, $J = 2.2$ Hz, H-8) and 6.69 (1H, *d*, $J = 2.2$ Hz, H-6).

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