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TWO NEW ISOFLAVONES FROM THE HEARTWOOD OF *CLADRASTIS LUTEA*

CLADRASTIN AND CLADRIN

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Abstract—The isolation and structural elucidation of the two new isoflavones cladrastin (I) and cladrin (XI) from *Cladrastis lutea* (Michx. f.) K. Koch are described. Other isoflavones also present are formononetin (XIV) and afrormosin (XV), both of which were previously known.

THE genus Cladrastis, which belongs to the family Leguminosae, is a small one with only five species. A limited amount of chemical research has been carried out on this genus in the past, the only relevant reference in the literature being to the isolation of the alkaloid cytisine from *C. Amurensis* Benth.[1] The one Cladrastis species native to the U.S. is *C. lutea* (Michx. f.) K. Koch, commonly called the yellowwood tree. It is nowhere abundant, and is probably the rarest of the American hardwood trees. As suggested by its common name, the tree possesses a beautiful golden yellow heartwood due to the presence of flavonoids. Color tests indicated that the heartwood was completely devoid of alkaloids.

TLC of the crude heartwood methanolic extracts on an eight inch silica gel plate ($CHCl_3$:MeOH 4:1) revealed the presence of about 23 compounds. However, 18 of these probably make up less than 5% of the extracts.

Several separation procedures were initially explored in order to obtain pure compounds. The method that finally led to the isolation of cladrastin (I), a compound that fluoresces blue under long-wavelength (3660 Å) UV light, consisted of percolating hot benzene through the chips of *C. lutea*. The extracts were then dried, and traces of impurities removed by treatment with small portions of ether. Final purification was effected by recrystallization from ethanol. Cladrastin (I), the colorless compound so obtained crystallized easily, m.p. 206–207°.

The UV spectrum of cladrastin (I) exhibited the characteristics of an isoflavone.[2] Additionally, color tests also indicated the isoflavone character of cladrastin; a yellow color appeared when the compound was added to aqueous sodium hydroxide, to conc sulfuric acid, or to magnesium-hydrochloric acid.[2]

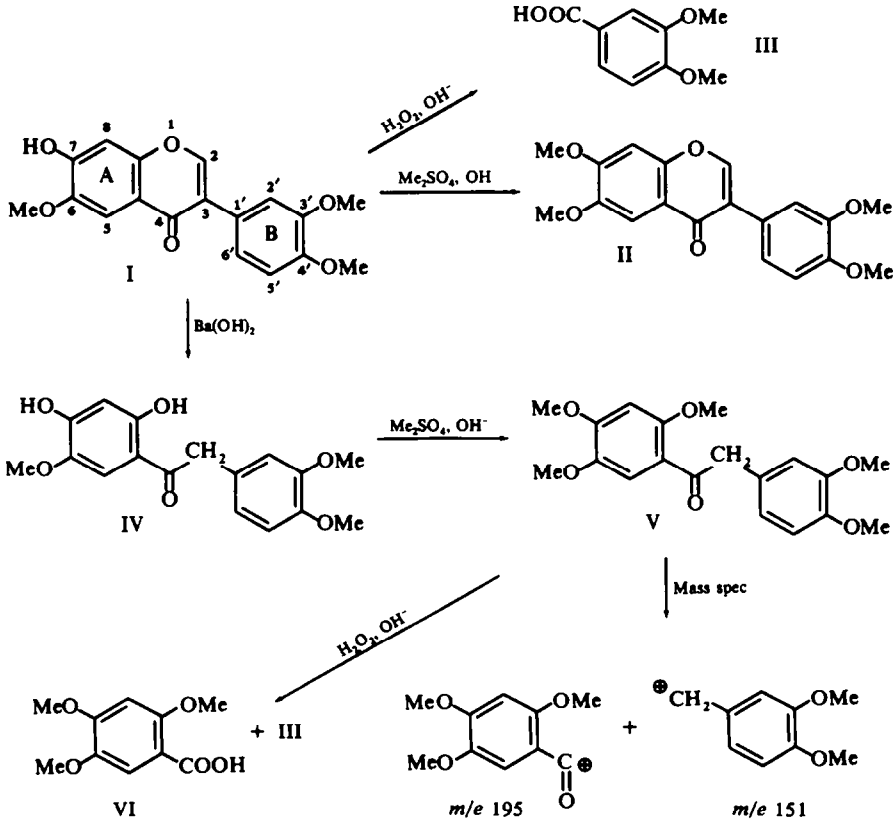
The IR spectrum in chloroform showed strong absorption in the 1620 cm^{-1} region characteristic of a conjugated CO function, and a band at 3532 cm^{-1} representing OH absorption.

The mass spectra of isoflavones is usually not too informative. In the case of cladrastin (I), the molecular ion appeared at *m/e* 328, $C_{18}H_{16}O_6$. Since two oxygens are incorporated within the isoflavone skeleton, cladrastin must possess four oxygenated substitutents.

The NMR spectrum in DMSO-d_6 exhibited absorption corresponding to three OMe groups, two of them at 3·82 δ and one at 3·92 δ. It was therefore clear at this stage that the four oxygenated substitutents of cladrastin were in the form of one OH and three OMe groups. These conclusions were strengthened by the fact that O-methylcladrastin (II), obtained through O-methylation of I exhibited four OMe absorptions at 3·93, 3·97, and 4·00 δ (2 OMe) in the NMR spectrum in $CDCl_3$.

Oxidation of an isoflavone with alkaline hydrogen peroxide is known to afford an acid derived from ring B, provided that this part of the isoflavone molecule is devoid of OH substitution. The acid that was isolated from cladrastin (I) by this procedure was 3,4-dimethoxybenzoic acid (III). Therefore the ring B substitution pattern in cladrastin had to consist of two OMe groups located at the 3′ and 4′ positions on the isoflavone skeleton.

SCHEME 1



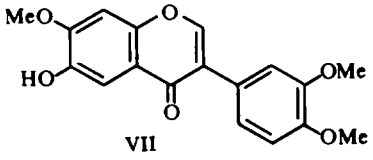
To gain more insight into the substitution pattern of cladrastin, the isoflavone was refluxed with 10% aqueous barium hydroxide to afford a crystalline and colorless

deoxybenzoin that gave a green ferric chloride test, and which was later formulated as IV. Treatment of this material with dimethyl sulfate furnished the new deoxybenzoin V which showed a negative ferric chloride test. The NMR spectrum of V indicated the presence of 5 OMe groups, and the mass spectrum exhibited a molecular ion at *m/e* 346 ($C_{19}H_{22}O_6$), and intense peaks at *m/e* 195 and 151 (Scheme I).

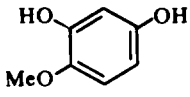
The fully methylated deoxybenzoin V also underwent basic hydrogen peroxide oxidation to yield two carboxylic acids which were separated by preparative TLC, and shown to be 2,4,5-trimethoxybenzoic acid (VI) and 3,4-dimethoxybenzoic acid (III). It follows that the substitution pattern for cladrastin must be 6,7,3′,4′, with an OH group at either C-6 or C-7, and OMe groups at the other positions.

The choice of structure I for cladrastin over the alternate expression VII, isocladrastin, where the OH and OMe groups at C-6 and C-7 are reversed, was reached on the basis of synthetic studies.

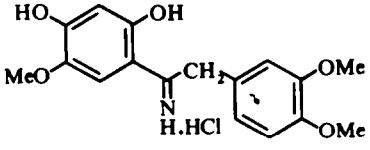
Hoesch condensation of 3,4-dimethoxybenzyl cyanide (VIII) with 4-methoxyresorcinol (IX) yielded the immonium hydrochloride X which upon hydrolysis afforded the desired deoxybenzoin IV. Synthetic compound IV was identical in all



VII



IX



X

respects with the same material derived through aqueous barium hydroxide treatment of cladrastin (I). Conversion of IV to cladrastin was then achieved by treatment with zinc cyanide and dry hydrogen chloride in anhydrous ether at 0°. The isoflavone so obtained was identical in all respects with naturally occurring cladrastin (I).

As a further check on the structural assignment for cladrastin, isocladrastin (VII) was also synthesized and found to be different from the naturally occurring product. The details of the synthesis of VII are described in the Experimental.

C. Lutea was also found to contain three additional isoflavones, and these were isolated by the following procedure. The benzene extracts of the plant were placed on preparative silica gel TLC plates, and ether was used as the eluent. Several plates had to be used because of the relative insolubility of the isoflavones in ether. By the alternate use of short (2537 Å) and long wavelength UV light, four bands could be detected. The slowest band (band 4) corresponded to cladrastin. Band 2 turned out to be the new isoflavone cladrin (XI). Bands 3 and 4 showed up as a blue coloration under long wave-length UV light, while the other two bands appeared as a very light violet tinge. All four bands showed up as dark strips under short wave-length light.

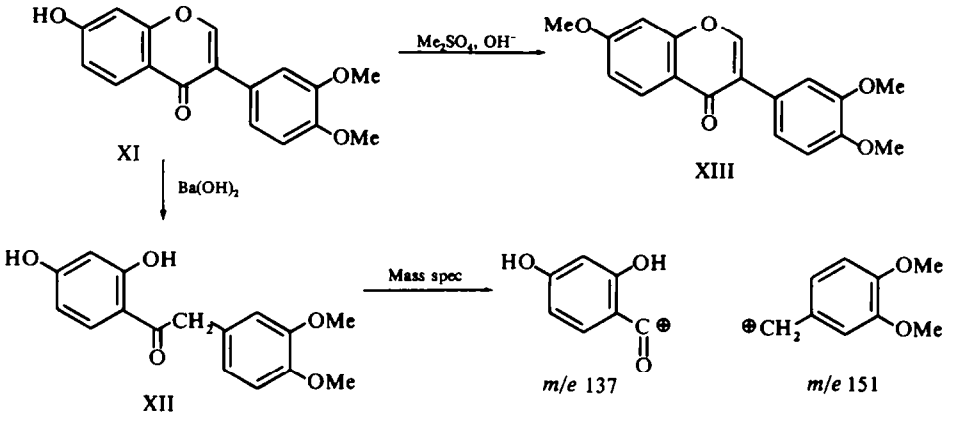
Cladrin (XI), mp 257–258°, is a colorless, crystalline isoflavone whose IR spectrum in Nujol showed a broad OH absorption at 3100 cm^{-1} and a conjugated CO at

1620 cm^{-1}. The NMR spectrum in trifluoroacetic acid showed the presence of two OMe groups superimposed at 4·07 δ.

The mass spectrum exhibited a molecular ion at *m/e* 298 ($C_{17}H_{14}O_5$), so that cladrin (XI) had three oxygenated substitutents, one as an OH group, and two in the form of OMe.

When cladrin (XI) was refluxed in aqueous barium hydroxide for 2 hr, a crystalline deoxybenzoin (XII) was obtained which gave a reddish-brown coloration with ferric chloride. Its mol wt was 288 as shown by mass spectrometry, with other intense peaks at *m/e* 151 and 137 (Scheme 2). Mass spectral analysis thus indicated that there were two OMe groups on ring B, and one OH on the A ring of the parent isoflavone cladrin (XI).

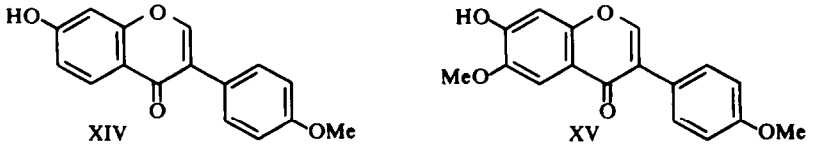
SCHEME 2



Since cladrin corresponds to a desmethoxycladrastin, it was possible at this stage to adumbrate that it is the C-6 OMe group of cladrastin (I) that is missing in cladrin, so that cladrin had to be represented by expression XI. Furthermore, methylation of cladrin (XI) furnished O-methylcladrin (XIII). A compound of structure XIII had acturally been found in nature as the isoflavone cabreuvin,[3] and indeed O-methylcladrin and cabreuvin were found to have indentical m.ps. But no cabreuvin was available for direct comparison.

Perusal of the literature then revealed that structure XI now being assigned to cladrin, although not previously found in nature, had acturally been synthesized.[4] In our hands, repetition of this synthesis then afforded cladrin (XI), identical in all respects with the natural product.

Going back now to the benzene extracts from *C. lutea*, the fraction that corresponded to the fastest moving band on TLC (band 1) was found to correspond to the known isoflavone formononetin (XIV).[5] Band 3 yielded afrormosin (XV), sometimes called afromosin, another known isoflavone.[6]



As could be expected, the TLC R_f values for the four isoflavones are in the order of their degree of substitution. Formononetin (XIV) is only disubstituted and has the largest R_f, while cladrastin (I) which is tetrasubstituted is the slowest moving material. Cladrin (XI) and afrormosin (XV), both of which are trisubstituted, have intermediate R_f values.

Isoflavones occur characteristically in the sub-family Papilionoideae,[7] and it is not surprising to find that *C. lutea* belongs to this division of the Leguminosae. The common feature of the four isoflavones of *C. lutea* is that an OH is always present at C-7, and a OMe is at C-4′.

EXPERIMENTAL

M.ps are uncorrected. The NMR spectra were obtained on a Varian A-60 unit. All TLC was on Merck Silica Gel GF_{254}; band fluorescence was observed with UVS11 or UVL22 lamps manufactured by Ultra-Violet Products, San Gabriel, California.

Isolation of cladrastin (I)

The initial extracts obtained from the percolation of hot benzene through 900 g of *C. lutea* chips for about 1 hr were set aside because of the high content of impurities. Percolation with fresh benzene for about another hr then resulted in the isolation of 0·8 g crude cladrastin, which still had traces of impurities as detected by TLC. The benzene was evaporated and the solid was treated with small portions of ether to remove most of the impurities. Several recrystallizations with EtOH then yielded pure cladrastin, m.p. 206–207°, as white crystals which gave a negative $FeCl_3$ reaction. The mass spectrum exhibited peaks at *m/e* 328 (M^+, $C_{18}H_{16}O_6$), 327 = $(M-1)^+$, 310 = $(M-18)^+$, and 283 = $(M-45)^+$; λ_{max}^{EtOH} 206, 220, 262 and 320 mμ (log ε 4·50, 4·42, 4·37 and 4·07); NMR DMSO-d_6, with TMS internal standard, H-2 8·32δ (s), H-2′ 6·96δ (s), H-5′ and 6′ broad band 7·02–7·16δ, H-8 7·25δ (s), and H-5 7·49δ (s). (Found: C, 65·95; H, 4·87. Calc. for $C_{18}H_{16}O_6$: C, 65·85; H, 4·9%).

Oxidation of cladrastin (I) *to* 3,4-*Dimethoxybenzoic acid* (III)

Into a soln of cladrastin (200 mg) in MeOH (20 ml) under reflux, KOH aq (0·25 g in 5 ml water) was added in small portions (1 ml every ½ hr), followed by the addition of small quantities of H_2O_2 (30%, 1 cc at a time, total 5 ml) in the course of 4 hr. The soln was tested occasionally to make certain it remained alkaline. After standing for 12 hr at room temp, the mixture was freed from alcohol, and water was added. The alkaline soln was extracted with ether, acidified, and again extracted with ether. The ethereal soln was passed through a silica gel column. Evaporation of the ether and repeated recrystallizations with benzene-hexane yielded 44 mg of the white crystalline compound III, m.p. 179–180° (reported 180–181°); identical with an authentic sample of 3,4-dimethoxybenzoic acid.

Deoxybenzoin 2,4-*dihydroxy*-5-*methoxyphenyl*-3,4-*dimethoxybenzyl ketone* (IV) *from cladrastin* (I)

Cladrastin (400 mg), $Ba(OH)_2$ (2·0 g), and water (18 ml) were refluxed for 2 hr under N_2. The mixture was acidified and extracted with ether to obtain 240 mg of the white crystalline ketone giving a green $FeCl_3$ reaction and having m.p. 166–167° upon recrystallization from EtOH. The mass spectrum exhibited a peak at *m/e* 318 (M^+, $C_{17}H_{18}O_6$). λ_{max}^{EtOH} 208, 233, 282 and 344 mμ (log ε 4·35, 4·20, 4·17 and 3·93). (Found: C, 63·64; H, 5·68. Calc. for $C_{17}H_{18}O_6$: C, 64·14; H, 5·70%).

2,4,5-*Trimethoxyphenyl*-3,4-*dimethoxybenzyl ketone* (V) *from* 2,4-*dihydroxy*-5-*methoxyphenyl*-3,4-*dimethoxybenzyl ketone* (IV)

2,4-Dihydroxy-5-methoxyphenyl-3,4-dimethoxybenzyl ketone (240 mg), Me_2SO_4 (5·0 ml), anhyd K_2CO_3 (5·0 g), and dry acetone (40 ml) were refluxed for 24 hr. The inorganic salts were removed, and the acetone evaporated from the filtrate. The residue was heated with NaOH aq for 10 min, and the solid collected and recrystallized from EtOH to give 230 mg of the white crystalline V, m.p. 121°. The mass spectrum exhibited peaks at *m/e* 346 (M^+, $C_{19}H_{22}O_6$), 151 = $(M-195)^+$, and 195 = $(M-151)^+$; λ_{max}^{EtOH} 207, 231, 270 and 325 mμ (log ε 4·30, 4·26, 4·02 and 3·84).

Oxidation of 2,4,5-*trimethoxyphenyl*-3,4-*dimethoxybenzyl ketone* (V) *to* 3,4-dimethoxybenzoic acid (III) *and* 2,4,5-*trimethoxybenzoic acid* (VI)

2,4,5-Trimethoxyphenyl-3,4-dimethoxybenzyl ketone (230 mg) in MeOH (20 ml) under reflux, was .reated with KOH aq and small quantities of H_2O_2 in a procedure similar to the oxidation of cladrastin. The reaction mixture separated into two spots on a silica gel plate (ether as eluent) as detected by fluorescence under the UVS 11 Mineralight lamp. One of the spots had the same R_f as authentic 3,4-dimethoxybenzoic acid, while the other spot had the same R_f as authentic 2,4,5-trimethoxybenzoic acid. The mixture of acids was therefore streaked onto an 8 in × 8 in silica gel plate which was developed with ether, then dried at room temp for about 10 min, and again eluted with ether. The IR spectrum of the content of the more mobile band showed the material to be 3,4-dimethoxybenzoic acid. The middle portion of the bottom band was treated with a mixture of $CHCl_3$ and MeOH to separate the acid from the silica gel. Evaporation of the solvent and recrystallization from benzene yielded 29 mg of a white crystalline VI, m.p. 142–143° (reported 144°);[8] whose IR spectrum was identical with that of authentic 2,4,5-trimethoxybenzoic acid.

Methylation of cladrastin (I) *to* O-*methylcladrastin* (II)

Cladrastin (100 mg), Me_2SO_4 (3·0 ml), anhyd K_2CO_3 (5·0 g), and dry acetone (40 ml) were refluxed for 24 hr. The inorganic salts were removed, and the acetone evaporated from the filtrate. The residue was heated with NaOH aq for 10 min, and the solid collected and recrystallized from EtOH, to give 80 mg of the white crystalline II, m.p. 187–188°. The mass spectrum exhibited peaks at *m/e* 342 (M^+, $C_{19}H_{18}O_6$), 341 = (M-1)$^+$, 327 = (M-15)$^+$, 311 = (M-31)$^+$, and 299 = (M-43)$^+$; λ_{max}^{EtOH} 208, 220, 263 and 317 mμ (log ε 4·39, 4·39, 4·33 and 3·97).

Synthesis of isocladrastin (VII)

Deoxybenzoin 2,5-*Dihydroxy*-4-*methoxyphenyl*-3,4-*dimethoxybenzyl ketone.* Methoxyquinol (7·8 g), 3,4-dimethoxybenzyl cyanide (9·4 g), anhyd $ZnCl_2$(5·0 g), and dry ether (40 ml) were saturated with HCl for 2 hr and set aside at 0° for 24 hr. The ether was decanted from the resultant solid, and the solid washed with dry ether and then refluxed with 50 cc water for 2 hr. The ppt was collected on a fritted glass funnel and washed with EtOH. Recrystallization from EtOH resulted in 2·7 g yellow crystals of deoxybenzoin, m.p. 188–189°. The mass spectrum exhibited peaks at *m/e* 318 (M^+, $C_{17}H_{18}O_6$), 167 = (M-151)$^+$, and 151 = (M-167)$^+$; λ_{max}^{EtOH} 206, 235, 279 and 352 mμ (log ε 4·40, 4·24, 4·08 and 3·87).

Isocladrastin (VII)

The above deoxybenzoin (½ g) was dissolved in 20 ml ethyl formate and the mixture dripped onto about 0·5 g powdered Na at −10°. After 24 hr below 0° and 48 hr at room temp, ice-water and 6N HCl was slowly added and the solid was collected on a fritted glass funnel and washed with small portions EtOH. Recrystallization from EtOH yielded 91 mg of a white crystalline compound, m.p. 256°. The mass spectrum exhibited peaks at *m/e* 328 (M^+, $C_{18}H_{16}O_6$), 313 = (M-15)$^+$, and 285 = (M-43)$^+$; λ_{max}^{EtOH} 207, 218, 263 and 326 mμ (log ε 4·48, 4·46, 4·37 and 4·00).

Synthesis of Cladrastin (I)

4-*Methoxyresorcinol* (IX). This was prepared by a procedure slightly different from that described by Crosby.[9] Performic acid [10] was made by carefully heating 50 ml 30% H_2O_2 with 250 ml 97% formic acid to 60°, followed by cooling to 0°. Isovanillin (30 g) was dissolved in 150 ml $CHCl_3$. By means of a dropping funnel, performic acid was slowly added to the aldehyde over a period of about 2 hr while maintaining the temp at 0°. The addition of performic acid was stopped as soon as an aliquot from the mixture showed the absence of isovanillin by TLC. The mixture was treated with $NaHSO_3$ and the formic acid was removed from the red colored soln in a flash evaporator. 3-Hydroxy-4-methoxyphenyl formate distilled at 119–125°/4 mm, yield 26·0 g of colorless crystals, m.p. 56° (reported 58°). Saponification of this ester then afforded 4-methoxyresorcinol.[9]

2,4-*Dihydroxy*-5-*methoxyphenyl*-3,4-*dimethoxybenzyl ketone* [*IV*]

A mixture of IX (11·4 g), VIII (20·0 g), anhyd $ZnCl_2$ (2·5 g), and dry ether (40 ml) was saturated with HCl for 2 hr, and set aside at 0° for 24 hr. The ether was decanted from the resultant solid, and the

solid washed with dry ether and then refluxed with 50 ml water for 2 hr. The mixture was cooled and then extracted with ether and $CHCl_3$. Evaporation of the solvent followed by recrystallization from EtOH resulted in 3·6 g. of white IV, m.p. 166–167°, giving a green $FeCl_3$ reaction. The mass spectrum exhibited ion peaks at *m/e* 318 (M^+, $C_{17}H_{18}O_6$), 167 = (M-151)$^+$, and 151 = (M-167)$^+$; λ_{max}^{EtOH} 208, 233, 282 and 344 mμ (log ε 4·35, 4·20, 4·17 and 3·93).

Synthetic cladrastin (I)

A mixture of 500 mg of IV, $Zn(CN)_2$ (0·50 g), and anhyd ether (20 ml) was saturated with dry HCl at 0° for 1·5 hr. The mixture was set aside for 48 hr at 0°. The ether was decanted, and the residual oil was washed with ether, then heated with water (20 ml) on a water-bath for 45 min. The mixture was cooled, and the solid was collected on a fritted glass funnel and washed with EtOH. Recrystallization from EtOH afforded 98 mg of the white crystalline I, m.p. 206–207°, identical in all respects with natural cladrastin.

Isolation of formononetin (XIV), *cladrin* (XI), *and afrormosin* (XV)

A portion of the original benzene extracts from *C. lutea* (170 mg) was dissolved in acetone, and streaked onto preparative TLC plates. The plates were developed with ether, dried, and again developed with ether. The bands were then collected and washed with acetone. The order of mobility from highest R_f to lowest was formononetin, cladrin, afrormosin and cladrastin. Each fraction was then rechromatographed as above. The products finally isolated were 27 mg of formononetin, m.p. 260°, 33 mg of cladrin, m.p. 257°; 51 mg of afrormosin, m.p. 229°; and finally 28 mg of cladrastin, m.p. 206°.

Cladrin (XI). Cladrin crystallized easily from EtOH; λ_{max}^{EtOH} 207, 217sh, 251sh, 260, and 290 sh mμ (log ε 4·34, 4·30, 4·29, 4·29 and 4·07); λ_{min}^{EtOH} 235 mμ (log ε 4·24).

2,4-*Dihydroxyphenyl*-3,4-*dimethoxybenzyl ketone* (XII) *from cladrin* (XI). Cladrin (20 mg) was refluxed in 20 ml 10% $Ba(OH)_2$ aq for 2 hr to yield 14 mg of XII, m.p. 182–183°.

Methylation of cladrin (XI) *to O-methylcladrin* (*cabreuvin*) (XIII). Cladrin was methylated with Me_2SO_4 as described above for cladrastin to afford O-methylcladrin, m.p. 165°; NMR $CDCl_3$ two MeO 3·88 δ, one MeO 3·92 δ, singlets.

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