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Studies on the Chemistry of Lichens

14 *. The Structure of Calycin

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Calycin has been shown to have the structure (*4*) and not, as previously supposed, the isomeric *o*-hydroxypulvinic dilactone structure (*1*). An improved synthesis of calycin is described.

The spectroscopic investigation of calycin methyl ether and its geometrical isomer furnished additional support for the proposed structure of calycin.

In 1880 Hesse [1] isolated a deep red compound, calycin, from the lichen *Lepraria candelaris* Shaer. About 50 years later Asano *et al.*[2] formulated calycin as *o*-hydroxypulvinic dilactone (*1*) on the grounds of degradative and synthetic experiments.

The high carbonyl frequencies of pulvinic dilactone (*3*) and related compounds (Table 1; *cf.* Ref.[3]) together with the easy opening of one of the lactone rings with formation of vulpinic acid (*11*) on short boiling with methanol, indicate that such lactone systems are highly strained.

Calycin has a lower carbonyl frequency (Table 1) and remains unchanged even after prolonged boiling with methanol. Either of the less strained structures (*4*) and (*6*) therefore seemed more reasonable for calcyin than (*1*). In order to study this question, an attempt was made to repeat the synthesis of calycin reported by Asano *et al.*[2]

o-Methoxybenzylcyanide was prepared in good yield by treating the corresponding benzylchloride in dimethyl sulphoxide solution with an excess of potassium cyanide at room temperature. When equimolecular amounts of *o*-methoxybenzylcyanide and diethyl oxalate were refluxed in ethanolic sodium ethoxide solution a low yield of ethyl 3-cyano-3-(*o*-methoxyphenyl)pyruvate was obtained. Subsequent condensation of this compound with benzylcyanide gave mainly 2,5-diphenyl-3,4-dioxoadiponitrile (*8*) instead of the expected 2-(*o*-methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile (*9*) which could only be isolated in poor yield by fractional crystallisation of the reaction product.

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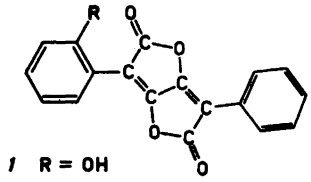
Table 1. Carbonyl stretching frequencies of calycin and pulvinic lactones.

Compound	ν_{CO} (cm^{-1}; in KBr)	
Calycin	1 790	1 775 1 715
Pulvinic dilactone	1 825	1 800 (sh)
o-Methoxypulvinic dilactone	1 805	1 780 (sh)
p-Methoxypulvinic dilactone	1 810	1 785 (sh)

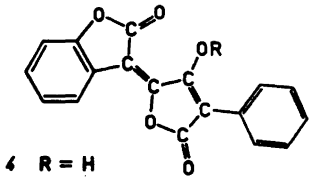
However, when sodium hydride in 1,2-dimethoxyethane was used as the base, both condensation steps could be accomplished at room temperature and high yields of the desired products were obtained.

Hydrolysis of the dinitrile (*9*) with sulphuric acid in acetic acid-water gave *o*-methoxypulvinic dilactone (*2*), m.p. 170—171°, and hydrolysis with hydrobromic acid-acetic acid yielded calycin, m.p. 248.5—249.5°, identical with an authentic sample isolated from *Lepraria candelaris* Shaer.

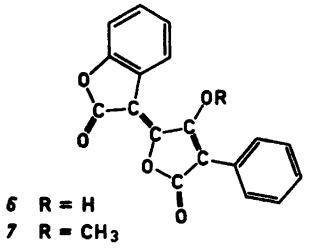
Vulpinic acid (*11*) is an enol of pronounced acidity and reacts readily with diazomethane giving a colourless methylether (*cf.* Ref.[4]). Calycin reacted similarly, affording an O-methyl derivative, m.p. 174—176°, which was not identical with *o*-methoxypulvinic dilactone (*2*). Structure (*1*) for calycin must therefore be incorrect and the true structure should be either (*4*) or (*6*). The low hydroxyl and carbonyl stretching frequencies of the crystalline calycin indicate strong hydrogen bonding. Intramolecular hydrogen bonds are possible in (4)



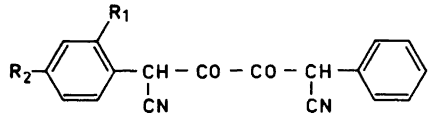
1 R = OH
2 R = OCH_3
3 R = H



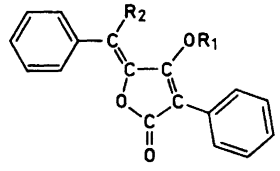
4 R = H
5 R = CH_3



6 R = H
7 R = CH_3



8 $R_1 = R_2 = H$
9 $R_1 = OCH_3$, $R_2 = H$
10 $R_1 = H$, $R_2 = OCH_3$



11 $R_1 = H$, $R_2 = COOCH_3$
12 $R_1 = CH_3$, $R_2 = COOCH_3$
13 $R_1 = R_2 = H$
14 $R_1 = CH_3$, $R_2 = H$

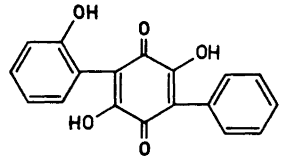


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Table 2. Infra-red absorption of the hydrogen bonded carbonyl groups of calycin, vulpinic acid and pulvinone.

Compound	ν_{CO} (cm^{-1}; in KBr)	ν_{CO}(cm^{-1}; in dioxan)	Shift (cm^{-1})
Calycin	1 715	1 722	7
Vulpinic acid (*11*)	1 685	1 682	−3
Pulvinone (*13*)	1 697	1 767	70

but in (6) only intermolecular hydrogen bonds can be formed. The type of hydrogen bonding present can be determined [5] by measuring the carbonyl frequencies of compounds in dioxan solution and in the pure state.

Calycin, vulpinic acid (*11*) and pulvinone (*13*) were investigated by this method. Of the two latter pulvinone can form only intermolecular hydrogen bonds. Since calycin and vulpinic acid showed very nearly the same infra-red carbonyl frequencies in dioxan solution and in the pure state (Table 2), both compounds must form intramolecular hydrogen bonds. By contrast a large difference was observed for pulvinone (Table 2), indicating strong intermolecular hydrogen bonding.

Ultraviolet absorption measurements supported these conclusions. Calycin and vulpinic acid exhibited large blue-shifts on methylation but pulvinone showed only a comparatively small shift (Table 3, *cf.* Ref.[5]). Calycin must therefore have the structure (*4*).

An isomeric monomethyl ether, m.p. 235—237°, together with unchanged starting material was isolated from the attempted mild hydrolysis of calycin methyl ether (*5*) with hydrochloric acid in dilute acetic acid. The isomer was also obtained in low yield by sublimation of calycin methyl ether at 0.001 mm. The great similarities in ultraviolet and infra-red absorption strongly suggest that the high melting "*iso*calycin methyl ether" is the geometrical isomer (*7*) of calycin methyl ether. From a closer examination of the absorption spectra of calycin methyl ether and *iso*calycin methyl ether additional indirect evidence for the structure of calycin was obtained.

In calycin methyl ether the absorption band originating from the conjugated double bond has a higher frequency and a lower apparent extinction coefficient (1 635 cm^{-1}, ε 240) than *iso*calycin methyl ether (1 625 cm^{-1}, ε 410). This indicates a *trans-trans* configuration for calycin methyl ether and *cis-trans* for the isomer (*cf.* Ref.[6]).

Inspection of models shows that the hydrogen atoms of the methoxyl group will probably interact with the benzene rings in both (*5*) and (*7*), but slightly less in (*5*). Since the conjugated systems are very similar, the corresponding ultraviolet absorption should occur at longer wavelength in the compound (*5*) than in (*7*) and have a slightly higher absorption coefficient in (*5*) [7,8]. In agree-

Table 3. Ultraviolet absorption of calycin, vulpinic acid and pulvinone and their methyl ethers.

Compound	Absorption (mμ; in dioxan)	Absorption of *O*-methyl derivative (mμ; in dioxan)	Shift (mμ)
Calycin	424	391	−33
Vulpinic acid	371	324	−47
Pulvinone	337	328	−9

ment with this calycin methyl ether absorbs at 391 mμ (ε 28 000) and the isomer at 384 mμ (ε 27 400).

A similar type of hydrogen-hydrogen interaction in diphenyl polyenes [9] has been shown to cause a splitting of the IR band at 690 cm^{-1} which is due to the C—H out of plane vibrations of *mono*substituted benzene rings (*cf.* Ref.[10]). Calycin, where no such interaction is possible, absorbs at 685 cm^{-1} whereas calycin methyl ether (695, 680 cm^{-1}) and *iso*calycin methyl ether (700 and 680 cm^{-1}) exhibit two bands. The apparent extinction coefficient of the higher of these frequencies is greater in calycin methyl ether (*5*) (695 cm^{-1}, ε 100) than in the isomer (*7*) (700 cm^{-1}, ε 45).

Finally an interesting difference in infra-red carbonyl absorption was observed for calycin methyl ether and its isomer. Both compounds contain two different carbonyl functions but calycin methyl ether showed only one carbonyl band (1 775 cm^{-1}) whereas *iso*calycin methyl ether had two (1 780, 1 760 cm^{-1}). A possible explanation is that the methoxyl group increases the coumaranone carbonyl frequency in calycin methyl ether (*5*) by a dipolar field effect similar to that observed for halogen atoms [11,12]. It was not possible, however, to show that the lower carbonyl frequency of the isomer (*7*) corresponded to the coumaranone carbonyl since the frequencies did not differ appreciably for the benzalcoumaranones (1 752—1 770 cm^{-1}) and the vulpinic acid derivatives (1 760—1 785 cm^{-1}) examined.

The relation between polyporic and pulvinic acid derivatives is obvious. Polyporic acid is converted to pulvinic lactone by oxidation with lead tetraacetate [13]. The close relationship between these compounds and calycin is also indicated by the fact that some species of *Stictae* contain both calycin, pulvinic lactone, and polyporic acid [14].

It seems probable that calycin is formed in nature by oxidation of the hypothetical *o*-hydroxypolyporic acid (*15*).

EXPERIMENTAL

Melting points were determined on a Kofler block and are corrected. Infra-red absorption spectra were taken on a Perkin-Elmer spectrophotometer No. 21, fitted with a sodium chloride prism. Ultraviolet absorption spectra were recorded with a Beckman DK-2 spectrophotometer.

o-*Methoxybenzylcyanide.* (a) *o*-Methoxybenzylchloride (120 g) in dimethylsulphoxide solution (1 l) was stirred for 18 h with potassium cyanide (250 g) at 25°, then 5 min at 100°. After cooling, water (2 l) was added and the product extracted with ether. Evaporation of the solvent, steam distillation and finally distillation at reduced pressure afforded pure *o*-methoxybenzylcyanide (100 g, 89 %), m.p. 70—71° (lit.[15] 68°).

(b) *o*-Methoxybenzylchloride (300 g) in dimethylformamide-water solution (3:1, 2 l) was stirred with potassium cyanide for 18 h at 25°, then 0.5 h at 100°. After the addition of water (2 l) the product was extracted with ether. Steam distillation and subsequent distillation at reduced pressure gave *o*-methoxybenzylcyanide (220 g, 78 %), m.p. 70—71°.

Ethyl 3-cyano-3-(o-methoxyphenyl)pyruvate. Sodium hydride (50 % in oil, 57 g) was added to a mixture of dry dioxan (800 ml) and 1,2-dimethoxyethane (200 ml) under an atmosphere of dry hydrogen. *o*-Methoxybenzylcyanide (80 g) and diethyl oxalate (120 g), containing a few drops of methanol were added all at once. After a while, the reaction started with violent evolution of hydrogen. The reaction-mixture was allowed to stand overnight at room temperature, then cautiously diluted with water (2 l) and washed with ether. On acidification with acetic acid ethyl 3-cyano-3-(*o*-methoxyphenyl)pyruvate slowly deposited as long needles (105 g, 78 %), m.p. 108—109° (from acetic acid-water; lit.[2] 104—105°).

2-(o-Methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile (9). (a) Sodium hydride (50 % in oil, 37 g) was added to a solution of benzylcyanide (28 g) in 1,2-dimethoxyethane (100 ml) under an atmosphere of dry, oxygen-free nitrogen. The mixture was cooled to −10° and ethyl 3-cyano-3-(*o*-methoxyphenyl)pyruvate (55 g) in 1,2-dimethoxyethane (200 ml) containing a trace of methanol, was slowly added. The cooling bath was then removed and the mixture allowed to stand overnight at room temperature. The solution was then diluted with water (1 l) and washed with ether. On acidification with acetic acid 2-(*o*-methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile deposited as a crystalline mass (58 g, 82 %), m.p. 233—237° (decomp.) (from acetic acid, lit.[2] 262°). (Found: OCH_3 9.3. Calc. for $C_{19}H_{14}N_2O_5$: OCH_3 9.7).

(b) Ethyl 3-cyano-3-(*o*-methoxyphenyl)pyruvate (5 g) and benzylcyanide (2.5 g) were refluxed for 2.5 h with sodium ethoxide [2] (from 2.4 g sodium in 45 ml ethanol). After cooling the solution was acidified with acetic acid and the precipitate collected. Fractional crystallisation from acetic acid gave 2,5-diphenyl-3,4-dioxoadiponitrile (*8*) (0.9 g), m.p. 263—267° (decomp.) (lit.[16] 270°) and small amounts of the reported reaction product, 2-(*o*-methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile (0.2 g), m.p. 232—236° (decomp.) [*cf.* (*a*)]. The identity of the compounds was confirmed by mixed melting point determination with authentic samples prepared according to procedure (*a*) and Ref.[16], respectively, and by comparison of the infra-red absorption spectra.

o-*Methoxypulvinic lactone* (*2*). 2-(*o*-Methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile (1.7 g) was refluxed for 1 h with a mixture of acetic acid (20 ml), sulphuric acid (conc. 10 ml), and water (15 ml). After cooling water was added (50 ml). The precipitate was collected, washed with water and dried. The product was refluxed for 15 min with acetic anhydride and then poured on to ice. *o*-Methoxypulvinic dilactone (1.5 g, 88 %) was obtained, m.p. 170—171° (from acetic acid, sublimed) (lit.[17] 172—173°). (Found: C 71.0; H 4.1; OCH_3 9.7. Calc. for $C_{19}H_{12}O_5$: C 71.2; H 3.8; OCH_3 9.7).

Calycin (4). 2-(*o*-Methoxyphenyl)-5-phenyl-3,4-dioxodiponitrile (22 g) was refluxed for 1.5 h with hydrobromic acid (48 %, 200 ml) in acetic acid (1 l). On cooling calycin separated out as red needles (10.4 g, 49 %), m.p. 249—249.5° (from acetic acid), undepressed on admixture with an authentic sample isolated from *Lepraria candelaris*. λ_{max} 208 mμ (ε 19 800), 237 mμ (ε 15 700), 253 mμ (ε 7 700), 430 mμ (ε 24 700) (in *cyclo*hexane), 422 mμ (ε 17 000) (in dioxan). (Found C 70.0; H 3.3. Calc. for $C_{18}H_{10}O_5$: C 70.6; H 3.3).

Calycin methyl ether (5). Calycin (7.4 g) in ether was treated with ethereal diazomethane, containing traces of methanol, for 15 min at room temperature. Excess diazomethane was destroyed with acetic acid, and unreacted calycin (0.8 g) filtered off. Evaporation of the ether solution gave calycin methyl ether (6.8 g, 98 %), m.p. 174—176° (from acetic acid). λ_{max} 205 mμ (ε 15 700), 254,5 mμ (ε 5 800), 396 mμ (ε 12 800) (in *cyclo*hexane), 391 mμ (ε 28 000) (in dioxan). (Found: C 71.2; H 3.9; OCH_3 9.2. Calc. for $C_{19}H_{12}O_5$: C 71.2; H 3.8; OCH_3 9.7).

"Iso*calycin methyl ether" (7),* (a) Calycin methyl ether was sublimed. The most volatile part consisted mainly of calycin methyl ether, but from the least volatile part of the sublimate an isomerised product, "*iso*calycin methyl ether", (5 mg), m.p. 235—237° (from acetic acid) (preheating to 225°) was obtained. λ_{max} 205 mμ (ε 15 000), 255 mμ (ε 5 100), 387 (ε 11 000) (in *cyclo*hexane), 384 mμ (ε 27 400) (in dioxan). (Found: C 71.2; H 4.1; OCH_3 9.75. Calc. for $C_{19}H_{12}O_5$: C 71.2; H 3.8; OCH_3 9.7).

(b) Calycin methyl ether (0.28 g) was refluxed for 2 h with hydrochloric acid (2 N, 1.5 ml) in acetic acid (30 ml). On cooling, a mixture of calycin methyl ether and the isomeric ompound (0.10 g) was obtained. Fractional crystallisation from acetic acid gave "*iso*calycin methyl ether" (0.025 g), m.p. 235—237°, undepressed on admixture with a sample obtained according to (*a*).

2-(p-Methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile (10) was made from ethyl 3-cyano-3-(*p*-methoxyphenyl)pyruvate [19] (5 g) and benzylcyanide (2.5 g) using the procedure described above for 2-(*o*-methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile. The yield was 5,3 g (83 %), m.p. 259—262° (decomp.) (lit.[18] 265°).

p-*Methoxypulvinic dilactone.* Hydrolysis of 2-(*p*-methoxyphenyl)-5-phenyl-3,4-dioxoadiponitrile (5 g) with sulphuric acid (12 ml) in acetic acid (25 ml) and water (18 ml) and subsequent refluxing of the product for 15 min with acetic anhydride furnished *p*-methoxypulvinic dilactone (3.6 g, 72 %), m.p. 200—201° (from acetic acid, followed by sublimation, lit. 196—198 [2], 184—186 [18]).

2,5-Diphenyl-3,4-dioxoadiponitrile (8) was prepared according to Volhard. M.p. 263—267° (decomp.) (lit.[16] 270°).

Pulvinic dilactone (3). Hydrolysis of the dinitrile (*8*) with dilute sulphuric acid (60 %)[16] gave pulvinic dilactone, m.p. 220—221° (lit.[20] 220—221°).

Vulpinic acid (11). Pulvinic dilactone (3 g) in dry methanol (50 ml) was refluxed for 0.5 h. The acid material was taken up in ether; evaporation of the solvent gave almost pure vulpinic acid (2.7 g, 81 %), m.p. 147—149° (from aqueous methanol, lit.[21] 148—149°), ν_{CO} 1 770 cm^{-1}.

O-Methylvulpinic acid (12). Vulpinic acid (1 g) on treatment for 0.5 h with ethereal diazomethane containing a trace of methanol gave O-methylvulpinic acid (1 g), m.p. 137—139° (from methanol, lit.[22] 141°), ν_{CO} 1 785 cm^{-1}.

Pulvinone (13) was prepared according to Claisen *et al.*[23] M.p. 247—249° (from acetic acid) (lit.[23] 248—249°).

o-Methylpulvinone (14). A suspension of pulvinone in ether was treated for 0.5 h with ethereal diazomethane containing a trace of methanol. Evaporation of the solvent gave *O*-methylpulvinone (1 g), m.p. 104—106° (from methanol, lit.[24] 104—105°) ν_{CO} 1 760 cm^{-1}.

4,6-Dimethoxy-3-benzalcoumaranone[25] (ν_{CO} 1 770 cm^{-1}), *4,6-dimethoxy-3-(4-methoxybenzal)coumaranone* (ν_{CO} 1 752 cm^{-1}) and *4,6-dimethoxy-3-(3,4,5-trimethoxybenzal)coumaranone* (ν_{CO} 1 755 cm^{-1}) were all kindly supplied by Professor J. Gripenberg.

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