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The Structure of Caloploicin, a New Lichen Trichloro-depsidone

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From a crustose lichen belonging to the genus *Caloplaca*, a new trichloro-depsidone named caloploicin (Ia) has been isolated in addition to a dichloro-depsidone: vicanicin (IIa) and four known anthraquinone derivatives: parietin (III), fallacinol (IV), fallacinal (V), and emodin (VI), and the structures Ia and IIa have been elucidated on the bases of chemical and physicochemical evidences. It has been suggested that caloploicin would be a common constituent of some species of the genus *Caloplaca*. As for vicanicin, it has been demonstrated that the accumulated evidences are in good accord with the revised structure (IIa) rather than the previously proposed one [E].

The lichens of the genus *Caloplaca* are remarkable by the yellow to red color, which has been said to be due to the anthraquinone pigments.[2] A crustose lichen belonging to the same genus grown on the rock along the stream of Settsu-kyō in Osaka prefecture exhibits a beautiful orange color. As a continuation of the investigation on the lichen phenolics,[3] we have undertaken the chemical study of the lichen metabolite[4] and isolated four known hydroxy-anthraquinone pigments and two chlorinated depsidones of which a trichloro-depsidone now named caloploicin (Ia) is a new substance. In the present paper, we wish to describe the details of the investigation.[5]

Lichen material was extracted with ether and acetone successively and the combined extract was chromatographed repeatedly with silica gel to afford parietin (III), fallacinol (IV), fallacinal (V), emodin (VI), caloploicin (Ia), and vicanicin (IIa, a dichloro-depsidone tentatively designated as Ca-II). Among the anthraquinone derivatives, parietin (III) is the major one (0.80% from the air-dried lichen material) and fallacinal (V) is the second (0.15%), while the other two were obtained only in a trace amount. They were identified with the authentic samples by direct comparison. As frequently experienced among the concurrent metabolites, III, IV, and V vary respectively with the oxidation stage of the alkyl side chain. Of two chloro-depsidones, caloploicin (Ia) was obtained in 1.78% yield and vicanicin (IIa) in 0.45% yield.

Caloploicin (Ia) shows the hydroxyl (3520 cm^{-1}) and ester carbonyl (1735, 1240 cm^{-1}) absorption bands in its infrared (IR) spectrum. It showed a positive Beilstein test and the molecular composition $C_{17}H_{13}O_5Cl_3$ was established by the elemental analysis and mass spectrum. Particularly the molecular ion peaks at *m/e* 402, 404, 406, and 408 with the approximate intensity ratio of 27: 27: 9: 1 clearly demonstrate the presence of three chlorine atoms in the molecule. The same isotope peak abundance of molecular ions is also observed in the mass spectra of following two derivatives, Ib and Ic.

On ordinary acetylation Ia yielded a monoacetate (Ib), whose infrared (IR) spectrum shows the presence of phenolic acetate (1785, 1180 cm^{-1}) in addition to the original ester

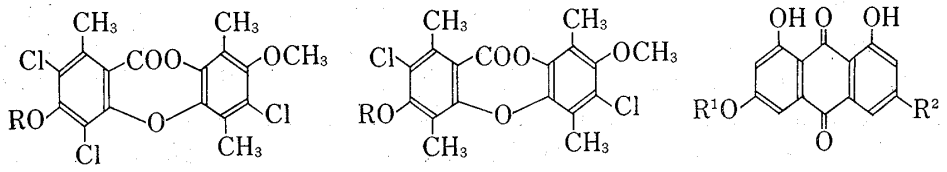
1) Location: *Toneyama, Toyonaka, Osaka.*
2) J. Santesson, *Phytochemistry*, **9**, 2149 (1970).
3) The preceding paper on the subjects: I. Yosioka, H. Yamauchi, K. Murata, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 1082 (1972).
4) The identification of the lichen has not yet been accomplished.
5) Preliminary report: I. Yosioka, K. Hino, M. Fujio, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1070 (1971).

carbonyl (1748, 1240 cm^{-1}). The proton magnetic resonance (PMR) spectrum of Ib shows four three-proton singlets at δ 2.35, 2.47, 2.57, and 2.64 ascribable to one phenolic acetate and three aromatic methyls and one three-proton singlet at δ 3.82 due to a methoxyl function.

Methylation of Ia with diazomethane afforded a monomethyl ether (Ic), which has been shown to possess three aromatic methyls and two methoxyls by the three-proton singlets at δ 2.30, 2.51, 2.62, 3.76, and 3.91 in its PMR spectrum. The IR spectrum of Ic shows the ester carbonyl absorption bands at 1754 and 1240 cm^{-1}.

Based on these evidences,[6] a paritial structure[A] (Chart 2) possessing a fully substituted depsidone framework has been advanced for caloploicin. Furthermore, since caloploicin possesses three aromatic methyl functions, the biogenetic consideration on the lichen depsidone via depside[7] as shown in Chart 2 has led to a presumption that caloploicin is formulated either as [B], [C], or Ia. The arrows denote the possible site of chlorination.

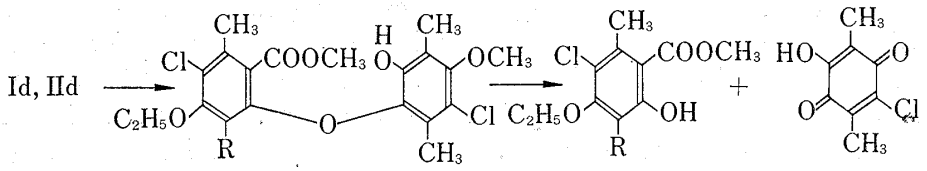
As for the structure elucidation of depsidone, there have been known several degradation procedures starting with methanolysis followed by such as: i) chlorination, stannous chloride reduction, and methanolysis;[8] ii) nitric acid oxidation in acetic acid or propionic acid;[8-10] iii) lead tetraacetate oxidation in acetic acid;[9] iv) sodium metaperiodate oxidation in acetic acid.[9] Among them, nitric acid oxidation has been adopted.



Ia : R=H caloploicin
Ib : R=CH_3CO
Ic : R=CH_3
Id : R=C_2H_5

IIa : R=H vicanicin (=Ca-II)
IIb : R=CH_3CO
IIc : R=CH_3
IId : R=C_2H_5

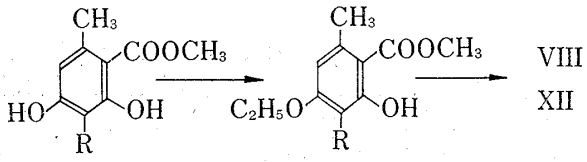
III : R^1=R^2=CH_3 parietin
IV : R^1=CH_3, R^2=CH_2OH fallacinol
V : R^1=CH_3, R^2=CHO fallacinal
VI : R^1=H, R^2=CH_3 emodin



VII : R=Cl
XI : R=CH_3

VIII : R=Cl
XII : R=CH_3

IX



Xa : R=H
methyl orsellinate

Xb : R=H

XIIIb : R=CH_3

XIIIa : R=CH_3
methyl β-orcinolcarboxylate

Chart 1

6) On the PMR assignment of depsidones: S. Huneck and P. Linscheid, *Z. Naturforsch.*, **23b**, 717 (1968).
7) *a*) S. Shibata, "Festschrift Kurt Mothes zum 65 Geburtstag," Gustav Fischer Verlag, Jena, 1965, p. 332; *b*) K. Mosbach, *Angew. Chem. intern. Edit.*, **8**, 240 (1969).
8) F.M. Dean, J.C. Roberts, and A. Robertson, *J. Chem. Soc.*, **1954**, 1432.
9) F.M. Dean, D.S. Deorha, A.D.T. Erni, D.W. Hughes, and J.C. Roberts, *J. Chem. Soc.*, **1960**, 4829.
10) S. Neelakantan, T.R. Seshadri, and S.S. Subramanian, *Tetrahedron*, **18**, 597 (1962).

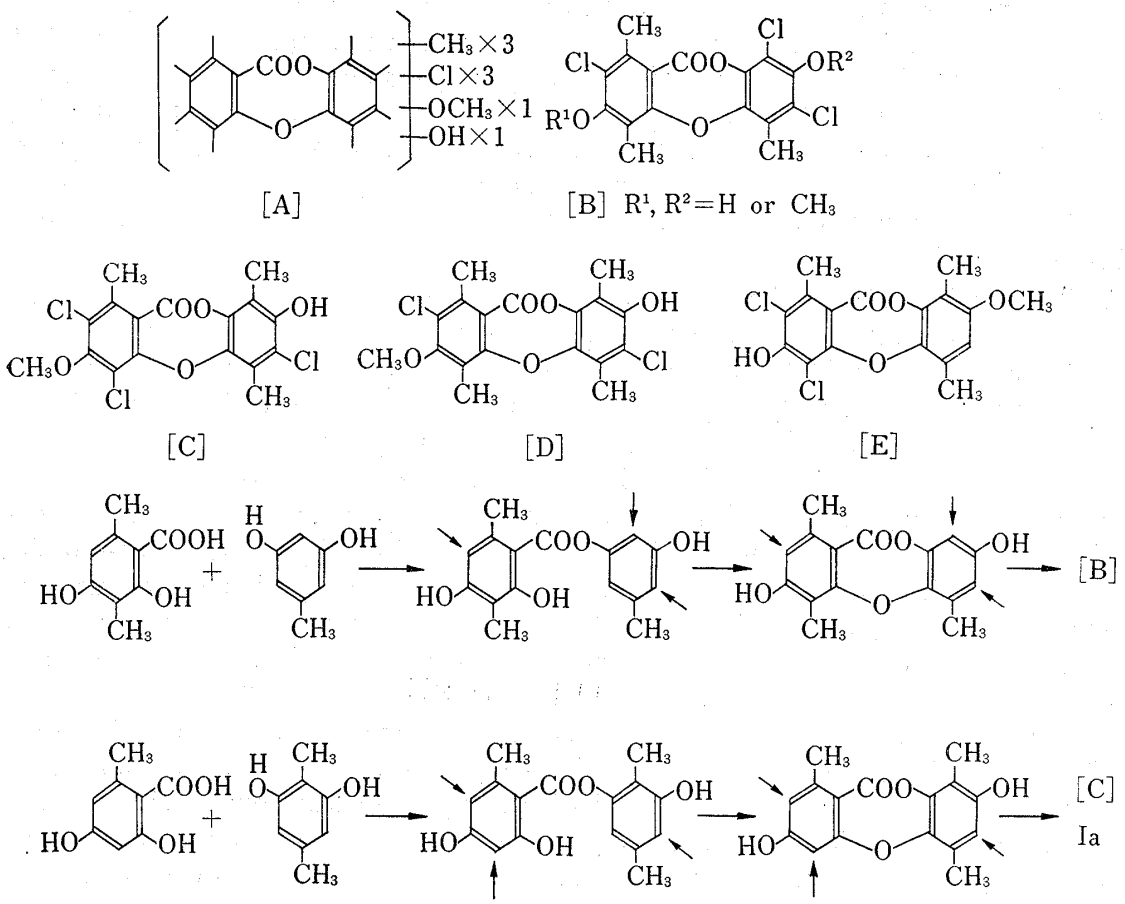


Chart 2

Treatment of Ia with ethyl iodide and potassium carbonate in acetone furnished an ethyl ether (Id), whose PMR spectrum shows the presence of a newly formed ethoxyl function by a three-proton triplet at δ 1.49 and a two-proton quartet at δ 4.16 along with the original three aromatic methyls and one methoxyl. Methanolic potassium hydroxide treatment of Id gave a hydroxy methyl ester (VII), whose structure has been corroborated by the spectral data. The IR spectrum of VII shows the hydroxyl (3550, 3260 (broad) cm^{-1}) and carbonyl (1737, 1708 cm^{-1})[11] absorption bands, while the PMR spectrum reveals the formation of a methyl ester (a three-proton singlet at δ 3.73 or 3.78) and a hydroxyl (a one-proton broad signal at δ 6.65, which is D_2O exchangeable) functions. Nitric acid oxidation of VII in glacial acetic acid furnished a colorless hydroxyl ester (VIII) and an orange yellow chloroquinone (IX). The molecular ion peaks at *m/e* 278, 280, and 282 with approximate relative intensity of 9: 6: 1 which agree with $C_{11}H_{12}O_4Cl_2$, a chelated ester carbonyl absorption band at 1668 cm^{-1}, an ethoxyl (a three-proton triplet (J=7 Hz) at δ 1.48 and a two-proton quartet (J=7 Hz) at δ 4.16), an aromatic methyl (a three-proton singlet at δ 2.49), a methoxyl (a three-proton singlet at δ 3.98), and a chelated hydroxyl (a D_2O exchangeable one-proton singlet at δ 11.44) singals in the PMR spectrum, all the physical properties have led to a reasonable formulation of the hydroxyl ester as VIII. The correctness of VIII has been established by the synthesis. Ethylation of methyl orsellinate (Xa) with ethyl iodide and potassium carbonate gave a monoethyl ether (Xb) which was then chlorinated with sulfuryl chloride to a dichloro derivative and the latter thus obtained was proved identical with the above hydroxy ester (VIII). On the other hand, the structure IX has been assigned to the chloroquinone on the basis of

11) The splitting would presumably be ascribed to the conformational isomerism of benzoate carbonyl function: C.J.W. Brooks, G. Eglinton, and J.F. Mormann, *J. Chem. Soc.*, **1961**, 106.

physical properties and the biogenetic consideration (especially concerning the allocation of two methyl functions). The mass spectrum of IX defines the molecular formula $C_8H_7O_3Cl$, the IR and ultraviolet (UV) spectra disclose a hydroxy-quinone property by the absorption bands at 3420, 1663 and 1653 cm^{-1}, and by the maxima at 274 nm (log ε: 4.23), 283 (4.29), and 410 (2.57), and the PMR spectrum shows the presence of two aromatic methyls by two three-proton singlets at δ 1.99 and 2.19 and that of a hydroxyl by a one-proton broad singlet at δ 7.05 which disappears on D_2O treatment. Consequently, the structure Ia has been established for caloploicin.

The minor depsidone designated as Ca-II (IIa) also showed a positive Beilstein test and the mass spectrum reveals the composition $C_{18}H_{16}O_5Cl_2$ particularly by the molecular ion peaks at *m/e* 382, 384, and 386 with the relative intensity of 9: 6: 1. The molecular ion peaks of the similar relative intensity are also observed in the mass spectra of following derivatives, IIb and IIc. The IR spectrum of IIa exhibits the hydroxyl (3520 cm^{-1}) and ester carbonyl (1730, 1260 cm^{-1}) absorption bands, while the PMR spectrum shows the presence of four aromatic methyls (two three-proton singlets at δ 2.33 and 2.45, and one six-proton singlet at δ 2.52), one methoxyl (a three-proton singlet at δ 3.78) and one hydroxyl (a one-proton broad signal at δ *ca.* 6.2). On acetylation with acetic anhydride and pyridine, IIa was converted to a monoacetate (IIb) whose IR and PMR spectra demonstrate the formation of a phenolic acetate function (1785, 1190 cm^{-1}, and a three-proton singlet at either one of 2.31, 2.36, 2.40 (3H each, singlet), and 2.50 (6H, singlet)). Methylation of IIa with diazomethane gave a monomethyl ether (IIc). Consequently, Ca-II has been elucidated to be a fully substituted depsidone derivative possessing four aromatic methyls, two chlorine, one methoxyl, and one hydroxyl, and considered to be constructed with two β-orcinolcarboxylic acid moieties via a depside intermediate.[7] Hereupon, two alternate structures, [D] and IIa, have become plausible for Ca-II, which possesses one more methyl and one less chlorine as compared with caloploicin (Ia).

To establish the structure IIa for Ca-II, the following degradations as for caloploicin (Ia) have been undertaken. Thus, an ethyl ether (IId), prepared from Ca-II with ethyl iodide and potassium carbonate in acetone, was treated with methanolic potassium hydroxide to afford a hydroxy methyl ester (XI). Nitric acid oxidation of XI in acetic acid furnished a colorless hydroxy ester and an orange chloroquinone (IX), the same compound derived from Ia. The former hydroxy ester has been proved to be methyl 5-chloro-4-O-ethyl-β-orcinolcarboxylate (XII) by the synthesis from methyl β-orcinolcarboxylate (XIIIa) *via* ethylation (giving XIIIb) followed by chlorination analogously as for the above described synthesis of VIII from Xa. Therefore, Ca-II has now been formulated as IIa, which coincides with the revised structure[12] of vicanicin, a dichloro-depsidone initially isolated from a lichen *Teloschistes flavicans* and to which the structure [E] being proposed by Neelakantan, *et al.*[10]

Although the direct comparison of Ca-II and vicanicin has been unavailable, the physical properties of both and their corresponding derivatives are alike (in lit.[10]: vicanicin, mp 248—250° (from benzene); methyl ether, mp 193—194° (from benzene); acetate, mp 213—214° (from AcOH)), and the evidences presented here are in good accord with the new formulation of vicanicin. Consequently, Ca-II has been concluded to be identical with vicanicin whose structure is formulated as IIa.

As for the mass spectra of caloploicin (Ia), vicanicin (IIa), and their derivatives, the examination of relative isotope peak abundance has provided the useful information on the chlorine content of the parent and fragment ion peaks as mentioned above and in the experi-

12) Confirmed by the X-ray analysis of the iodoacetate. J.R. Dyer, A.C. Baillie, V.M. Balthis, and J.A. Bertrand, Abstracts of Papers presented at the Southern Regional Meeting of the American Chemical Society, Atlanta, Georgia, U.S.A., Nov. 1—3, 1967. Cited in C.F. Culberson, "Chemical and Botanical Guide to Lichen Products," The Univ. of North Carolina Press, 1968, p. 165.

mental section. In addition, a common fragment ion peak of C_8H_9OCl[13] (*m/e* 156 and 158 with an approximate intensity ratio of 3: 1) has been observed in the mass spectra of Ia, Ib, Ic, IIa, IIb, and IIc. The ion at *m/e* 156, which appears with the intensity of 43—67% relative to the base peak, has been presumed to be derived from the right half aromatic moiety of the depsidones, although the exact structure has not been depicted.[14]

Several chlorinated depsidones have been known to occur as the fungal and lichen metabolites. They are gangaleoidin,[15a] diploicin,[15b] pannarin,[15c] nidurin,[8,9] and nornidurin.[8,9] Caloploicin and vicanicin are the first and second examples of the chloro-depsidones elucidated from the lichen of the genus *Caloplaca*. Finally, it seems to be noteworthy to point out that an unidentified trichloro substance, which has been noticed to give a molecular ion peak at *m/e* 402 and to distribute in some *Caloplaca* species as reported by Santesson in his extensive phytochemical investigation,[2] might be identical with caloploicin (Ia).

Experimental[16]

Isolation of Anthraquinones and Depsidones——Air-dried lichen materials (33.21 g), collected in fall 1966 at Settsu-kyō in Osaka prefecture, were extracted with ether (300 ml each, four times) and acetone (300 ml each, two times) successively at reflux (24 hr each). The ether extract weighed 1.896 g while the acetone extract weighed 288 mg after removing the less soluble portion by filtration. Most of the depsidones were obtained from the ether extract and the anthraquinones were from both. Repeated silica gel (Merck) column chromatography of the combined extracts eluting with *n*-hexane–benzene, benzene, and benzene–$CHCl_3$ afforded the following ingredients. The eluate with *n*-hexane–benzene (5: 1—3: 1) was crystallized from benzene and $CHCl_3$–acetone to give parietin (III, 264 mg, 0.80%) as the orange needles of mp 205°. Diacetate (prepared with acetic anhydride and pyridine), mp 194—195° (from benzene–*n*-hexane). Identification was made by direct comparison (thin-layer chromatography (TLC), mixed mp, and IR) with authentic parietin and its diacetate. The eluate with *n*-hexane–benzene (3: 1) was crystallized from benzene and from acetone to give fallacinal (V, 54 mg, 0.15%) as the orange needles of mp 259.5—260.5°, which was identified with the authentic sample as above. The eluate with *n*-hexane–benzene (2: 1—1: 1) was crystallized from benzene to give vicanicin (=Ca-II, IIa, 150 mg, 0.45%) as the colorless needles of mp 239—240°. The eluate with *n*-hexane–benzene (1: 1) and benzene was crystallized from benzene to give caloploicin (Ia, 593 mg, 1.78%) as the colorless needles of mp 260.5°. The combined eluate with benzene and benzene–$CHCl_3$ (1: 1) was further purified by preparative TLC developing with benzene–ether (1: 1) and $CHCl_3$–MeOH (40: 1) to afford a small amount of emodin (VI) and fallacinol (IV), which were further purified by sublimation. Emodin, mp 265—265.5° and fallacinol, mp 246—247°, were identified by direct comparison (TLC, mixed mp, and IR) with the authentic specimens.

Caloploicin (Ia)——Colorless needles from benzene, mp 260.5°. *Anal.* Calcd. for $C_{17}H_{13}O_5Cl_3$: C, 50.58; H, 3.25; Cl, 26.35. Found: C, 50.40; H, 3.48; Cl, 26.35. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1735, 1578, 1455, 1400, 1240, 1200, 1122; ν_{max}^{Nujol} cm^{-1}: 3300, 1700, 1580, 1545, 1408, 1253, 1175, 1135, 1088, 985, 800. UV λ_{max}^{EtOH} nm (log ε): 226 (inflex., 4.51), 258 (inflex., 3.89), 323 (3.79). Mass Spectrum *m/e*: 402 (19%), 404 (19%), 406 (6%), 408 (1%) (M^+); 367 (100%), 369 (68%), 371 (10%) (M^+-Cl); 374 (M^+-CO, 11%),[17] 352 (M^+-Cl-CH_3, 23%),[17] 339 (M^+-CO-Cl, 18%);[17] 156 (45%), 158 (12%) (C_8H_9OCl). High resolution Mass Spectrum: Calcd. for $C_{17}H_{13}O_5{}^{35}Cl_3$: 401.9822. Found: 401.9828; Calcd. for $C_{17}H_{13}O_5{}^{35}Cl_2{}^{37}Cl$: 403.9792. Found: 403.9799; Calcd. for $C_8H_9O^{35}Cl$: 156.0342. Found: 156.0358.

Caloploicin Monoacetate (Ib)——Acetylation of caloploicin (Ia, 34 mg) with acetic anhydride (1 ml) and pyridine (2.5 ml) by keeping overnight at 28°, followed by ordinary work-up and crystallization from benzene, afforded the mono acetate (Ib, 27 mg), mp 230—231.5°. *Anal.* Calcd. for $C_{19}H_{15}O_6Cl_3$: C, 51.20; H, 3.39; Cl, 23.87. Found: C, 51.33; H, 3.41; Cl, 23.42. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1785, 1748, 1460, 1445, 1422, 1397. 1368,

13) The composition was also confirmed by the high resolution mass spectrometry (see experimental section).
14) For the mass spectra of depsides and depsidones: S. Huneck, C. Djerassi, D. Becher, M. Barber, M. von Ardenne, K. Steinfelder, and R. Tümmler, *Tetrahedron*, **24**, 2707 (1968).
15) *a*) Y. Asahina and S. Shibata, "Chemistry of Lichen Substances," Japan Society for the Promotion of Science, Tokyo, Japan, 1954, p. 123; *b*) *Idem, ibid.*, p. 127; *c*) *Idem, ibid.*, p. 146.
16) The following instruments were used for the physical data. Melting points: Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and recorded as read; IR Spectra: Hitachi IR Spectrometers EPI-2 and EPI-G31; PMR Spectra (in $CDCl_3$): Hitachi H-60 and Varian A-60 NMR Spectrometers; UV Spectra: Shimadzu MPS-50L UV Spectrometer; Mass Spectra: Hitachi RMU-6D Mass Spectrometer.
17) These peaks (based on ^{35}Cl) are accompanied by the satelite isotope peaks of reasonable relative abundance, but they are omitted.

1240, 1200, 1180, 1075, 990, 890, 860. Mass Spectrum *m/e*: 444 (22%), 446 (21%), 448 (7.5%), 450 (1.5%) (M^+); 409 (31%), 411 (21%), 413 (4%) (M^+-Cl); 367 (100%), 369 (70%), 371 (17%) (M^+-CH_2CO-Cl); 416 (M^+-CO, 9%);[17] 402 (M^+-CH_2CO, 6%);[17] 374 (M^+-CH_2CO-CO, 15%);[17] 352 (M^+-CH_2CO-Cl-CH_3, 17%);[17] 156 (43%), 158 (15%) (C_8H_9OCl).

Caloploicin Monomethyl Ether (Ic)——To a solution of Ia (100 mg) in benzene (40 ml) was added excess ethereal diazomethane and the mixture was left standing for 3 hr and treated as usual. The product was crystallized from benzene–*n*-hexane to give colorless needles (90 mg) of mp 228—228.5°. *Anal.* Calcd. for $C_{18}H_{15}O_5Cl_3$: C, 51.76; H, 3.62; Cl, 25.47. Found: C, 51.46; H, 3.51; Cl, 25.64. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1754, 1457, 1440, 1403, 1385, 1240, 1120, 1085, 950. Mass Spectrum *m/e*: 416 (23%), 418 (23%), 420 (8%), 422 (1%) (M^+); 381 (100%), 383 (67%), 385 (11%) (M^+-Cl); 388 (M^+-CO, 16%),[17] 366 (M^+-Cl-CH_3, 25%),[17] 353 (M^+-Cl-CO, 23%);[17] 156 (43%), 158 (15%) (C_8H_9OCl).

Caloploicin Monoethyl Ether (Id)——A mixture of Ia (700 mg), dry acetone (60 ml), ethyl iodide (7 ml), and dry K_2CO_3 (7 g) was refluxed for 4 hr and filtered to remove insoluble inorganic material. The feathery crystals (Id) obtained by concentration of the filtrate were collected by filtration. The mother layer was evaporated and the $CHCl_3$ soluble portion was crystallized from acetone to give an additional crop of Id. Totally 717 mg of monoethyl ether (Id), mp 217—218°, was obtained. *Anal.* Calcd. for $C_{19}H_{17}O_5Cl_3$: C, 52.86; H, 3.97; Cl, 24.64. Found: C, 53.16; H, 3.94; Cl, 24.22. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1756, 1446, 1405, 1397, 1239, 1120, 990. PMR (δ): 1.49 (3H, t, J=7 Hz), 4.16 (2H, q, J=7 Hz) (OCH_2CH_3), 2.31, 2.50, 2.62, 3.77 (3H each, s, $CH_3\times3$, OCH_3).

Methanolysis of Id giving Hydroxy Methyl Ester (VII)——A suspension of Id (650 mg) in MeOH (60 ml) and 5% KOH–MeOH (10 ml) was stirred under ice-cooling for 1 hr to give a clear solution, which was acidified with dil. HCl and the resulting white precipitate was collected by filtration, washed and dried. Since purity of the product (660 mg) was shown by TLC, it was subjected to the following HNO_3 oxidation. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3260 (broad), 1737, 1708, 1578, 1454, 1402, 1327, 1284 (broad), 1130, 1093, 1066. PMR (δ): 1.45 (3H, t, J=7 Hz), 4.10 (2H, q, J=7 Hz) (OCH_2CH_3), 2.17 (6H, s), 2.31, 3.73, 3.78 (3H each) ($CH_3\times3$, $OCH_3\times2$), 6.65 (1H, br, $W_{h/2}$=8 Hz, D_2O exchangeable) (OH).

Oxidative Degradation of VII giving VIII and IX——To a solution of VII (660 mg) in glacial AcOH (50 ml) was added conc. HNO_3 (0.3 ml) and the total mixture was stirred for 20 min under ice-cooling, poured into water (300 ml) and extracted with ether three times (300 ml each). The combined ether solution was first washed with aq. $NaHCO_3$ until the aqueous layer became neutral. The ether layer was then extracted again with aq. $NaHCO_3$ (200 ml each, 4 times) and the pretty purple aqueous layer was taken, acidified with dil. HCl, extracted with ether and the ether extract was treated in a usual manner. Evaporation of the solvent followed by crystallization from *n*-hexane furnished an orange yellow needles (IX, 28 mg), of mp 124—125°. *Anal.* Calcd. for $C_8H_7O_3Cl$: C, 51.49; H, 3.78; Cl, 19.00. Found: C, 51.33; H, 3.81; Cl, 17.45. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3420, 1663, 1653, 1606, 1390, 1364, 1290, 1210, 1132, 1060, 895. UV $\lambda_{max}^{isopentane}$ nm (log ε): 274 (4.23), 283 (4.29), 410 (2.57). PMR (δ): 1.99, 2.19 (3H each, s) ($CH_3\times2$), 7.05 (1H, br, $W_{h/2}$=5 Hz, D_2O exchangeable). Mass Spectrum *m/e*: 186 (100%), 188 (36%) (M^+).

The remaining ether layer after extracting IX with aq. $NaHCO_3$ was washed with water, dried, and evaporated to give a product, which was purified by preparative TLC (Merck SiO_2HF_{254}, developing with ether–*n*-hexane (2: 5)) and recrystallized from H_2O–MeOH to afford colorless needles (VIII, 148 mg) of mp 87.5—88°. *Anal.* Calcd. for $C_{11}H_{12}O_4Cl_2$: C, 47.33; H, 4.33; Cl, 25.41. Found: C, 47.63; H, 4.25; Cl, 25.37. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1668, 1585, 1440, 1390, 1308, 1230, 1099. Mass Spectrum *m/e*: 278 (29%), 280 (18%), 282 (6%) (M^+); 246 (73%), 248 (47%), 250 (12%) (M^+-CH_3OH); 218 (100%), 220 (66%), 222 (14%) (M^+-CH_3OH-CO). The compound obtained here was identified (TLC, mixed mp, and IR) with the synthetic specimen prepared from methyl orsellinate (Xa) as described below.

Methyl 4-O-Ethylorsellinate (Xb)——A solution of methyl orsellinate (Xa, 200 mg) in dry acetone (20 ml) was treated with ethyl iodide (2 ml) and dry K_2CO_3 (2 g) and the total mixture was refluxed for 4 hr and filtered after cooling. The residue left by evaporation of the filtrate was treated with $CHCl_3$ and the soluble portion was crystallized from H_2O–MeOH to give colorless needles (157 mg) of mp 49—50°. *Anal.* Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.58; H, 6.66. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1656, 1620, 1582, 1445, 1329, 1257, 1204, 1110, 1050.

Methyl 3,5-Dichloro-4-O-ethylorsellinate (VIII)——To a solution of Xb (50 mg) in benzene (2 ml) was added freshly distilled SO_2Cl_2 (16 drops) and the mixture was refluxed for 1 hr and was added with an additional amount (12 drops) of SO_2Cl_2 and refluxed again for further 30 min. After evaporating *in vacuo* the resulting product was purified by preparative TLC (Merck SiO_2HF_{254}, ether–*n*-hexane (2: 1)) and crystallized from H_2O–MeOH to give colorless needles (20 mg) of mp 87.5—88°. The crystals thus prepared were identified (TLC, mixed mp, and IR) with VIII obtained by the HNO_3 oxidation of VII described above.

Vicanicin (=Ca-II) (IIa)——Colorless needles from benzene. mp 239—240°. *Anal.* Calcd. for $C_{18}H_{16}O_5Cl_2$: C, 56.41; H, 4.21; Cl, 18.50. Found: C, 56.56; H, 4.52; Cl, 18.53. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1730, 1600, 1575, 1455, 1400, 1260, 1140, 1110, 1080. UV λ_{max}^{EtOH} nm (log ε): 226 (inflex., 4.50), 270 (3.89), 325 (3.38). Mass Spectrum *m/e*: 382 (23%), 384 (15%), 386 (3%) (M^+); 347 (100%), 349 (37%) (M^+-Cl); 354 (M^+-CO, 27%);[17] 332 (M^+-Cl-CH_3, 41%);[17] 319 (M^+-Cl-CO, 20%);[17] 156 (62%), 158 (26%) (C_8H_9OCl).

Vicanicin Monoacetate (IIb)——Acetylation of IIa (21 mg) with acetic anhydride (1 ml) and pyridine (2.5 ml) followed by usual work-up and crystallization from benzene–*n*-hexane afforded colorless plates (IIb, 16.3 mg) of mp 211—211.5°. *Anal.* Calcd. for $C_{20}H_{18}O_6Cl_2$: C, 56.48; H, 4.27; Cl, 16.67. Found: C, 56.58; H, 4.34; Cl, 16.65. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1785, 1752, 1455, 1440, 1405, 1260, 1190, 1140, 1080, 970. PMR (δ): 2.31, 2.36, 2.40, 3.77 (3H each, s), 2.50 (6H, s) ($CH_3\times4$, $OCH_3\times1$, $OCOCH_3\times1$). Mass Spectrum *m/e*: 424 (24%), 426 (16%), 428 (2.5%) (M^+); 389 (58%), 391 (22%) (M^+-Cl); 347 (100%), 349 (36%) (M^+-Cl-CH_2-CO); 396 (M^+-CO, 25%);[17] 382 (M^+-CH_2CO, 5%);[17] 354 (M^+-CH_2CO-CO, 16%);[17] 339 (M^+-CH_2CO-CO-CH_3, 13%);[17] 332 (M^+-CH_2CO-Cl-CH_3, 25%);[17] 319 (M^+-CH_2CO-Cl-CO, 20%);[17] 156 (63%), 158 (20%) (C_8H_9OCl).

Vicanicin Monomethyl Ether (IIc)——Treatment of IIa (20 mg) with excess ethereal diazomethane followed by crystallization from benzene–*n*-hexane afforded colorless needles of mp 191—192° in a quantitative yield. Purity of the product was assured by TLC. Mass Spectrum *m/e*: 396 (25%), 398 (15%), 400 (2.5%) (M^+, $C_{19}H_{17}O_5Cl_2$); 361 (100%), 363 (40%) (M^+-Cl); 368 (M^+-CO, 28%);[17] 346 (M^+-Cl-CH_3, 31%);[17] 333 (M^+-Cl-CO, 21%);[17] 156 (67%), 158 (18%) (C_8H_9OCl).

Vicanicin Monoethyl Ether (IId)——A solution of IIa (53 mg) in dry acetone (5 ml) was treated with ethyl iodide (0.5 ml) and dry K_2CO_3 (0.5 g) and the total mixture was refluxed for 4 hr and filtered after cooling. Concentration of the filtrate by evaporation *in vacuo* afforded colorless feathery crystals of mp 196—197°. *Anal.* Calcd. for $C_{20}H_{20}O_5Cl_2$: C, 58.69; H, 4.93; Cl, 17.33. Found: C, 58.37; H, 5.11; Cl, 17.01. IR ν_{max}^{KBr} cm^{-1}: 1735, 1440, 1415, 1270, 1160, 1090, 1010.

Methanolysis of IId giving Hydroxy Methyl Ester (XI)——A mixture of IId (45 mg), MeOH (4 ml), and 5% KOH–MeOH (1 ml) was stirred at room temperature for 45 min and the resulting clear solution was acidified with dil. HCl and poured into water. The precipitate collected by filtration was washed with water and dried to give colorless powder (XI, 48 mg) which showed a single spot on TLC. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530, 1730, 1700, 1408, 1316, 1275, 1100.

Oxidative Degradation of XI giving XII and IX——To a solution of XI (40 mg) in glacial AcOH (4 ml) was added conc. HNO_3 (3 drops) and the total mixture was stirred at room temperature for 20 min and treated similarly as for the oxidative degradation of VII described above. The 5% $NaHCO_3$ soluble portion afforded an orange yellow quinone (mp 123°, crystallized from *n*-hexane), which was identified with IX by TLC, mixed mp, and IR. The product obtained from the ether layer was purified by preparative TLC and crystallized from H_2O–MeOH to give colorless needles (9 mg) of mp 64—64.5°, which was identified (TLC, mixed mp, and IR) with methyl 5-chloro-4-O-ethyl-β-orcinolcarboxylate (XII) prepared from methyl β-orcinolcarboxylate (XIIIa) described below. Mass Spectrum *m/e*: 258 (14.5%), 260 (4%) (M^+, $C_{12}H_{15}O_4Cl$); 226 (88%), 228 (29%) (M^+-CH_3OH); 198 (24%), 200 (9%) (M^+-CH_3OH-CO); 182 (100%), 184 (36%).

Methyl 4-O-Ethyl-β-orcinolcarboxylate (XIIIb)——A solution of methyl β-orcinolcarboxylate (XIIIa, 35 mg) in dry acetone (4 ml) was treated with ethyl iodide (0.4 ml) and dry K_2CO_3 (0.4 g) and the total mixture was refluxed for 2 hr and filtered after cooling. The residue left by evaporation of the filtrate was treated with $CHCl_3$ and the soluble portion was purified by preparative TLC and crystallized from H_2O–MeOH to afford colorless needles (XIIIb, 35 mg) of mp 119—120°. *Anal.* Calcd. for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.18; H, 7.07. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1654, 1610, 1578, 1440, 1395, 1300, 1275, 1230, 1195, 1160, 1135, 1035. Mass Spectrum *m/e*: 224 (M^+).

Methyl 5-Chloro-4-O-ethyl-β-orcinolcarboxylate (XII)——To a solution of XIIIb (25 mg) in benzene (2 ml) was added freshly distilled SO_2Cl_2 (20 drops) and the total mixture was refluxed for 1 hr and evaporated *in vacuo* to dryness. The product was then purified by preparative TLC (Merck SiO_2HF_{254}, ether–*n*-hexane (1: 1)) and crystallized from H_2O–MeOH to give colorless needles (27 mg) of mp 64—65°. *Anal.* Calcd. for $C_{12}H_{15}O_4Cl$: C, 55.71; H, 5.84; Cl, 13.71. Found: C, 55.40; H, 5.75; Cl, 13.94. The product obtained here was identical with XII obtained by the HNO_3 oxidation of XI described above.

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