[Chem. Pharm. Bull. 24(10)2340—2344(1976)]

UDC 547.914.4.02 : 581.192

On the Constituents of the Lichen in the Genus *Menegazzia*. Menegazziaic Acid, a New Depsidone from *Menegazzia asahinae* (Yas. *ex* Zahlbr.) Sant. and *Menegazzia terebrata* (Hoffm.) Mass

Teruhisa Hirayama, Fukujiro Fujikawa,[1a] Itiro Yosioka, and Isao Kitagawa[1b]

Kyoto College of Pharmacy[1a] and Faculty of Pharmaceutical Sciences, Osaka University[1b]

(Received January 12, 1976)

Following the thin–layer chromatographic examinations of the acetone extractive of two lichens: *Manegazzia asahinae* (Yas. *ex* Zahlbr.) Sant. and *M. terebrata* (Hoffm.) Mass, which have been disclosed to contain the same kinds of metabolites, a new depsidone named menegazziaic acid (**4**) has been isolated from *M. terebrata* in addition to stictic acid (**1**), atranorin (**2**), constictic acid (**3**), and chloroatranorin (**5**) The structure of menegazziaic acid has been established as **4**, in which the formyl function at C-3 of stictic acid (**1**) is replaced by a hydroxyl, on the basis of chemical and physicochemical evidence including the chemical conversion of menegazziaic acid to stictic acid (**1**).

The occurrence of stictic acid (**1**) and atranorin (**2**) in the lichens of the genus *Menegazzia* (previously included in the genus *Parmelia*) has been well documented.[2] In 1968, in the thin–layer chromatographic (TLC) survey of some kinds of lichens, Asahina pointed out[3] the presence of another depsidone named constictic acid (**3**), which often appears concomitantly with stictic acid (**1**) in some lichens of the genera such as *Lobaria*, *Usnea*, *Sphaerophorus*, and *Parmelia* (including *Menegazzia*) and is found without stictic acid (**1**) in some lichens of *Usnea* species. Later on, the structure of constictic acid has been elucidated as **3**.[4]

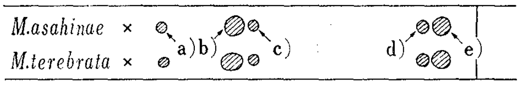


Fig. 1. TLC Diagram of the Acetone Extractives of the Lichens in the Genus *Menegazzia*

developing solvent: benzene-AcOEt-HCOOH=5:3:0.3
detection with: anisaldehyde-sulfuric acid
a) constictic acid (**3**), b) stictic acid (**1**)
c) menegazziaic acid (**4**), d) chloroatranorin (**5**)
e) atranorin (**2**)

As a continuative study on the lichen constituents,[5] we have examined by TLC the metabolites of two lichens *Menegazzia asahinae* (Yas. *ex* Zahlbr.) Sant. (syn. *Parmelia asahinae* Yas. *ex* Zahlbr.) and *M. terebrata* (Hoffm.) Mass (syn. *Parmelia pertusa* Schaer., *M. pertusa* (Schrank) B. Stein), and have noticed as shown in Fig. 1 that both lichens contain a new depsidone now named menegazziaic acid (**4**) (spot c) in addition to the known metabolites: stictic acid (**1**) (spot b), atranorin (**2**) (e), constictic acid (**3**) (a), and chloroatranorin (**5**) (d). This paper provides the chemical and physicochemical evidence which is consistent with assigning the structure **4** for menegazziaic acid.

The lichen materials (*Menegazzia terebrata*) was extracted with ether and acetone successively. The ether extractive, after silica gel column chromatography, gave atranorin (**2**)

---

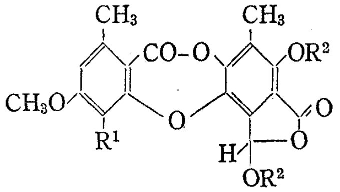
1) Location: *a) Yamashina-misasagi, Higashiyama-ku, Kyoto, 607, Japan; b) 133–1, Yamada-kami, Suita, Osaka, 565, Japan.*

2) C.F. Culberson, "Chemical and Botanical Guide to Lichen Products," The University of North Carolina Press, Chapel Hill, North Carolina, U.S.A., 1967, p. 391.

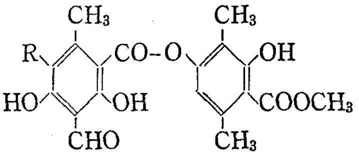
3) Y. Asahina, *Journ. Jap. Bot.*, 43, 97 (1968).

4) I. Yosioka, Y. Morita, and K. Ebihara, *Chem. Pharm. Bull.* (Tokyo), 18, 2364 (1970).

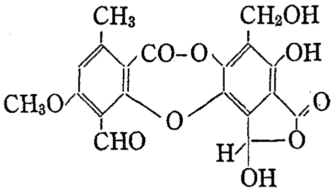
5) Preceding paper on the lichen constituents: T. Hirayama, F. Fujikawa, I. Yosioka, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), 24, 1602 (1976).



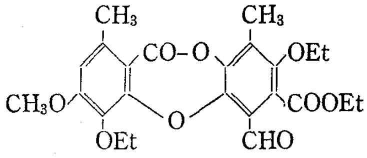
	R^1	R^2	
1	CHO	H	stictic acid
1a	CHO	Ac	
1b	$CH(OAc)_2$	Ac	
4	OH	H	menegazziaic acid
4a	OAc	Ac	
4b	OH	Ac	



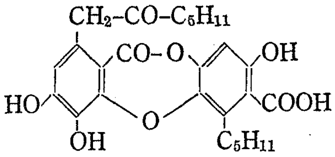
R=H atranorin (**2**)
R=Cl chloroatranorin (**5**)



constictic acid (**3**)



6



oxyphysodic acid (**7**)

Chart 1

and chloroatranorin (**5**) while from the acetone extractive were obtained stictic acid (**1**), constictic acid (**3**), and menegazziaic acid (**4**).

Menegazziaic acid (**4**) exhibited the positive coloration with the ferric chloride reagent (violet) and the aniline reagent (pale yellow).[6] The infrared (IR) spectrum of menegazziaic acid shows the absorption bands at 3400 (br) (hydroxyl), 1766, 1727 (depsidone, lactol), and 1619 cm^{-1} (aryl double bond). The close resemblance of menegazziaic acid to stictic acid (**1**)[7] has been shown by the proton magnetic resonance (PMR) spectra of both (Table I) in which the significant difference is that the former lacks the signal due to the formyl proton found in the latter (δ 10.50, s). The elemental and mass spectral analyses have shown that menegazziaic acid possesses one carbon less molecular composition than stictic acid (**1**).

TABLE I. PMR Data of Stictic Acid (**1**), Menegazziaic Acid (**4**), and Their Acetates (**1a**, **4a**) (δ values at 90 MHz in d_6-DMSO)[a]

	Ar-$C\underline{H}_3$	OAc	$OC\underline{H}_3$	Ar-$\underline{H}$	$C\underline{H}O$	$-\overset{|}{C}\underline{H}-OR^2$
1	2.21 2.51	—	3.93	7.07	10.50	6.62
4	2.17 2.34	—	3.84	6.78	—	6.78
1a	2.24 2.52	2.14 2.38	3.97	7.13	10.29	7.34
4a	2.24 2.49	2.12 2.28 2.38	3.86	7.08	—	7.54

[a] All signals are observed as singlets.
d_6-DMSO=hexadeuterodimethylsulfoxide

---

[6] Y. Asahina and S. Shibata, "Chemistry of Lichen Substances," Japan Society for the Promotion of Science, Tokyo, 1954, p. 10.

[7] S. Huneck and P. Linscheid, *Z. Naturforsch.*, 23b, 717 (1968).

Stictic acid diacetate (**1a**), which was initially prepared by alkaline treatment of **1** (hydrolysis of the depsidone ester linkage giving stictinic acid) followed by acetylation with acetic anhydride and pyridine (simultaneous reformation of the depsidone ring),[8] has been found to be readily prepared by direct acetylation of **1** with acetic anhydride and pyridine. On the other hand, treatment of **1** with acetic anhydride and conc. sulfuric acid has been known to furnish the tetraacetate (**1b**) in which the parent formyl function has suffered the acetal formation.[9] However, menegazziaic acid (4) was converted to the same triacetate (**4a**) under the both acetylation conditions. The comparison of the PMR spectra of **1a** and **4a** also shows the close resemblance of both compounds except that the formyl proton signal in **1a** is not observed in **4a** while the spectrum of **4a** shows one more phenolic acetoxyl signal than that of **1a** (Table I).

In addition to the above described evidence, the mass fragmentation pattern of menegazziaic acid in comparison with that of stictic acid (1)[10] supports a presumption that the structure of menegazziaic acid is expressed as 4 in which the formyl function at C-3 of stictic acid (1) is replaced by a hydroxyl. Thus, the fragment ions at *m/e* 356 (**i**, M^+-H_2O) (base peak) and *m/e* 328 (**ii**, M^+-H_2O-CO) are analogously formulated as in the case of **1**. The other prominent ions at *m/e* 181, 180, 179, and 151 are respectively formulated as **iii**, **iv**, **v**, and **vi** (tentative) either derived from the A part or from the B part upon the electron impact of **4** (M^+: *m/e* 374).

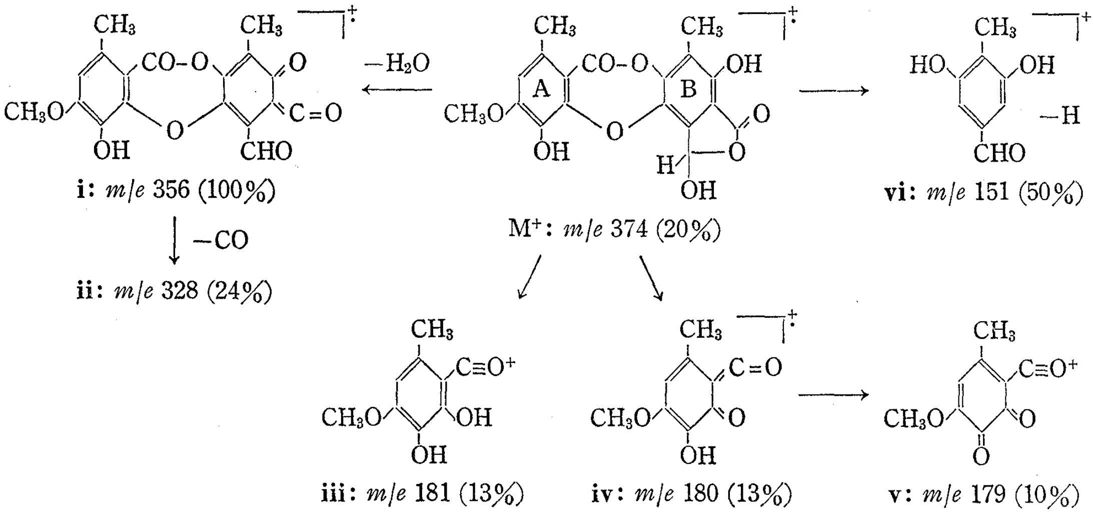


Chart 2. Mass Fragmentation of Menegazziaic Acid (4)

Finally, in order to chemically correlate menegazziaic acid (4) with stictic acid (1), several reactions have been undertaken. The first attempt was the alkaline hydrogen peroxide oxidation of **1** expecting the direct conversion of the formyl function to a hydroxyl,[11] but the attempt was not realized probably due to instability of the lactol moiety in **1** under the reaction conditions. Next, stictic acid diacetate (**1a**) was treated with *m*-chloroperbenzoic acid to furnish a phenolic diacetate (**4b**) in a moderate yield. Acetylation of the diacetate (**4b**) with acetic anhydride and conc. sulfuric acid gave a triacetate which has been found identical with menegazziaic acid triacetate (**4a**) in all respects, thus establishing that the structure of menegazziaic acid is formulated as **4**.

---

[8] *a*) F.H. Curd and A. Robertson, *J. Chem. Soc.*, **1935**, 1379; *b*) Y. Asahina, M. Yanagita, and I. Yosioka, *Chem. Ber.*, **69**, 1370 (1936).

[9] Y. Asahina, M. Yanagita, and T. Okami, *Chem. Ber.*, **66**, 943 (1933).

[10] S. Huneck, C. Djerassi, D. Becher, M. Barber, M. von Ardenne, K. Steinfelder and R. Tümmler, *Tetrahedron*, **24**, 2707 (1968).

[11] W.M. McLamore, *J. Am. Chem. Soc.*, **73**, 2225 (1951).

As for the presence of the lactol moiety in menegazziaic acid (**4**), ethylation of **4** with ethyl iodide and silver carbonate was undertaken to furnish an aldehyde (**6**) (colored deep yellow by the aniline reagent⁶⁾) which affords an additional evidence.

Menegazziaic acid (**4**) is the second example of depsidone possessing a pyrogallol-type moiety in the left aromatic ring, the first one being oxyphysodic acid (**7**) which was found to occur in most of the lichens belonging to the genus *Hypogymnia*.¹²⁾ It seems to be appropriate to mention that menegazziaic acid (**4**) is a unique depsidone by the possession of an oxygenated orcinol-type aromatic ring in the left half and a β-orcinol-type aromatic ring in the right half of its molecule.

Experimental¹³⁾

Isolation of Depsides and Depsidones——Air-dried lichen materials (150 g), collected at Mt. Ōmine in Nara prefecture, were extracted with ether twice (400 ml each for 48 hr) at room temperature and then extracted with acetone (200 ml) for 6 hr using a Soxhlet extractor. The ether extractive (2.2 g) was chromatographed on a silica gel (Merck, 70—230 mesh, 100 g) column eluting with benzene–AcOEt (9: 1). The first eluting depside was crystallized from acetone to give atranorin (**2**, 1 g, 0.66%) as colorless needles of mp 196°, which was identified with the authentic sample by direct comparison (mixed mp, TLC, and IR ($CHCl_3$)). The second eluate, on crystallization from acetone, gave chloroatranorin (**5**, 530 mg, 0.35%) as colorless needles of mp 208°, which was identified with the authentic sample by direct comparison as above. The acetone extractive (6 g) was chromatographed on a silica gel (Merck, 70—230 mesh, 150 g) column eluting with benzene–AcOEt–HCOOH (10: 3: 0.3). The first eluting depsidone was crystallized from aq. dioxane to give menegazziaic acid (**4**, 240 mg, 0.16%) as colorless fine crystals of mp 272—273°. *Anal.* Calcd. for $C_{18}H_{14}O_9$: C, 57.76; H, 3.77. Found: C, 57.83; H, 3.51. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3400 (br) (OH), 1766, 1727 (depsidone, lactol), 1619 (aryl C=C). Mass Spectrum *m/e* (%): 300 (**ii**$-$CO, 48%), 285 (**ii**$-CH_3$, 14%), 272 (**ii**$-$CO$\times$2, 32%). 244 (**ii**$-$CO$\times$3, 14%), and as given in Chart 2. PMR (d_6-DMSO) δ: as given in Table I. The second eluting substance was crystallized from acetone to give stictic acid (**1**, 3.3 g, 2.2%) as colorless fine crystals of mp 268—269° (decomp.), which was identified by direct comparison (mixed mp, TLC, and IR (KBr)) with the authentic sample. The third eluate was further purified by preparative TLC developing with benzene–AcOEt–HCOOH (5: 3: 0.3) to afford constictic acid (**3**, 300 mg, 0.2%), which was crystallized from aq. dioxane to furnish a pure sample of constictic acid, mp 198°. Constictic acid was identified with an authentic sample isolated from the lichen *Usnea aciculifera* VAIN. by direct comparison as above.

Menegazziaic Acid Triacetate (4a)——Acetylation of **4** (50 mg) with acetic anhydride (4 ml) and conc. H_2SO_4 (one drop) at room temperature followed by usual work-up and crystallization from aq. dioxane afforded **4a** (41 mg) as colorless needles of mp 222°. *Anal.* Calcd. for $C_{24}H_{20}O_{12}$: C, 57.60; H, 4.03. Found: C, 57.15; H, 4.26. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1770 (br), 1750 (sh) (depsidone, lactol, OAc), 1609 (aryl C=C). Mass Spectrum *m/e* (%): 500 (M^+, 3.5), 458 (M^+-CH_2CO, 4), 416 ($M^+-CH_2CO\times2$, 3), 398 ($M^+-CH_2CO\times2-H_2O$, 95), 374 ($M^+-CH_2CO\times3$, 4), 356 ($M^+-CH_2O\times3-H_2O$, 100), 328 (38%), 300 (50%), 272 (17%), 151 (46%). PMR (d_6-DMSO) δ: as given in Table I.

Acetylation of 4 with Acetic Anhydride and Pyridine giving 4a——A solution of **4** (10 mg) in pyridine (1 ml) was treated with acetic anhydride (0.5 ml), and the total mixture was left standing at room temperature overnight. The usual work-up of the product followed by crystallization from aq. dioxane afforded **4a** being identical with the above sample on TLC and mixed mp determination.

Stictic Acid Diacetate (1a)——Acetylation of **1** (350 mg) with acetic anhydride (5 ml) and pyridine (4 ml) followed by usual work-up and crystallization from aq. acetone afforded **1a** as colorless needles (320 mg) of mp 236°. *Anal.* Calcd. for $C_{23}H_{18}O_{11}$: C, 58.68; H, 3.90. Found: C, 59.16; H, 3.83. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1775 (br), 1736, 1703 (depsidone, lactol, OAc), 1605 (aryl C=C). Mass Spectrum *m/e* (%): 470 (M^+, 1), 428 (M^+-CH_2CO, 28), 410 (M^+–CH_3COOH, 27), 386 (M^+–$CH_2CO\times2$, 33), 368 (M^+–$CH_2CO\times2-H_2O$, 100). PMR (d_6-DMSO) δ: as given in Table I.

Oxidation of Stictic Acid Diacetate (1a) with *m*-Chloroperbenzoic Acid——A solution of **1a** (100 mg) in $CHCl_3$ (10 ml) was treated with a solution of *m*-chloroperbenzoic acid (100 mg) in $CHCl_3$ (5 ml), and the total mixture was left standing at room temperature overnight and washed with 10% $Na_2S_2O_3$, 10% KI, 10% $NaHCO_3$, and water successively. Usual work-up of the $CHCl_3$ solution followed by purification by column chromatography (Merck silica gel PF_{254}) eluting with benzene–dioxane–AcOH (12: 2: 0.2) afforded a white powder, which was crystallized from aq. dioxane to give **4b** (48 mg) as colorless fine crystals of mp 261°. *Anal.* Calcd. for $C_{22}H_{18}O_{11}$: C, 57.64; H, 3.96. Found: C, 57.39; H, 4.11. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3560 (OH), 1775 (br), 1750

---

12) T. Hirayama, F. Fujikawa, I. Yosioka, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **24**, 1596 (1976).

13) Instruments used in the experimental section and the experimental conditions for chromatography were same as in our previous paper.¹²⁾

(sh) (depsidone, lactol, OAc), 1624 (aryl C=C). Mass Spectrum *m/e* (%): 458 (M^+, 3), 416 (M^+-CH_2CO, 4), 398 (M^+-CH_3COOH, 25), 374 ($M^+-CH_2CO\times2$, 29), 356 ($M^+-CH_2CO\times2-H_2O$, 100). PMR (d_6-DMSO) δ: 2.11 (3H, s, OAc), 2.21 (3H, s, Ar–$C\underline{H}_3$), 2.37 (6H, s, Ar–$C\underline{H}_3$ & OAc), 3.86 (3H, s, $OC\underline{H}_3$), 6.83 (1H, s, Ar–$\underline{H}$), 7.53 (1H, s, $-\overset{|}{C}\underline{H}$–OAc).

Acetylation of 4b giving 4a——Acetylation of **4b** (20 mg) with acetic anhydride (2 ml) and conc. H_2SO_4 (one drop) followed by usual work-up and crystallization from aq. dioxane afforded **4a** (18 mg) as colorless needles of mp 222°, which was identified with above menegazziaic acid triacetate (**4a**) by mixed mp, TLC, and IR (KBr).

Ethylation of Menegazziaic Acid (4) giving 6——A solution of **4** (100 mg) in acetone (5 ml) was treated with ethyl iodide (5 ml) and silver carbonate (200 mg), and the total mixture was refluxed in a water bath for 24 hr. After cooling, the reaction mixture was filtered and evaporation of the filtrate gave a light yellow residue which was subjected to column chromatography (Merck silica gel PF_{254}) eluting with benzene–AcOEt (2:1). The pale yellow crystals thus obtained were recrystallized from MeOH to give **6** (73 mg) as colorless needles of mp 57°. *Anal.* Calcd. for $C_{24}H_{26}O_9$: C, 63.87; H, 5.72. Found: C, 63.61; H, 6.02. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1732 (br), 1695 (depsidone, CHO, $COOC_2H_5$), 1603 (aryl C=C). Mass Spectrum *m/e* (%): 458 (M^+, 100). PMR ($CDCl_3$) δ: 1.34 (6H, t, J=7 Hz, $OCH_2C\underline{H}_3\times2$), 1.40 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 2.31, 2.47 (3H each, both s, Ar–$C\underline{H}_3\times2$), 3.87 (3H, s, $OC\underline{H}_3$), 3.91, 4.13, 4.37 (2H each, all q, J=7 Hz, $OC\underline{H}_2CH_3\times3$), 6.61 (1H, s, Ar–$\underline{H}$), 10.80 (1H, s, $C\underline{H}O$).

Acknowledgement The authors are grateful to the late Professor Emeritus Y. Asahina and Dr. M. Nuno for the identification of the lichen materials.