UDC 582.29 : 581.19

161. Shoji Shibata and Hsüch-Ching Chiang*1 : Grayanic Acid, A New Lichen Depsidone.

(*Faculty of Pharmaceutical Sciences, University of Tokyo**1)

Asahina and Simosato[1] isolated a crystalline acid, m.p. 185°, from *Cladonia Grayi* MERRILL giving a molecular formula, $C_{21}H_{22}O_7$, and named grayanic acid. At that time, further investigation was not developed due to the shortage of material. The recent advancement of microchemical and modern physical methods in the investigation of natural products prompts us to reinvestigate the chemical structure of this lichen substance.

Grayanic acid, m.p. 186~189°, extracted from *Cladonia Grayi* MERRILL collected at Yoshida-Kenmaruo, Mt. Fuji, was employed for the present study. The analytical figures and the result of molecular weight determination (by Rast's method) showed that the molecular formula of grayanic acid should be amended to $C_{23}H_{26}O_7$, which involved 1 CH_3O. A phenolic carboxylic acid structure was suggested by the solubility in bicarbonate solution, the bluish violet coloration with ferric chloride and the yellow coloration with diazonium reagent.

The infrared spectrum of grayanic acid proved the presence of chelated carboxyl group (1650 cm^{-1} (Nujol, KBr, $CHCl_3$)), lactonic linkage, (1750 cm^{-1} (Nujol, KBr, $CHCl_3$)) and benzenoid ring structure (1570, 1610 cm^{-1} (Nujol, KBr, $CHCl_3$)).

Grayanic acid formed mono-acetate, $C_{25}H_{28}O_8$, m.p. 155~157°, which showed apparently the presence of phenolic acetate (1780 cm^{-1} (Nujol)) and non-bonded carboxyl (1695 cm^{-1} (KBr); 1705 cm^{-1} (Nujol)). Thus the carboxyl group was proved as being hydrogen bonded with the *ortho* phenolic hydroxyl. By the action of diazomethane, grayanic acid yielded a methyl ether methyl ester, $C_{25}H_{30}O_7$, m.p. 88~90°. The negative homofluorescein reaction[2] and the stability for methanolysis in boiling with methanol for 18 hours, suggested that grayanic acid is not a depside but a depsidone. This was actually proved by the formation of dicarboxylic acid, $C_{23}H_{28}O_8$, m.p. 168~170°, named grayanoldicarboxylic acid, which would be resulted by the cleavage of lactone linkage, and was shown as having *meta* situated two hydroxyls by the violet color with ferric chloride and the immediate change of coloration from red to faint yellow by the action of bleaching powder.

According to Hale,[3] the lichen depsides and the depsidones of orcinol and β-orcinol types can be distinguished each other by the characteristic ultraviolet absorption spectral curves. Referring Hale's result, the ultraviolet spectral maxima of grayanic

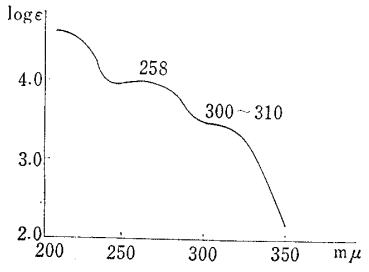


Fig. 1.

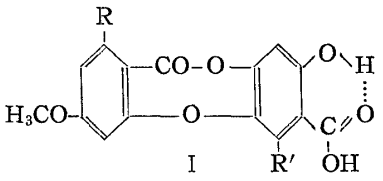
*1 Hongo, Tokyo (柴田承二, 江雪 卿).

1) Y. Asahina, Z. Simosato : Jap. J. Bot., **15**, 469 (1939).
2) cf. Y. Asahina, S. Shibata : "Chemistry of Lichen Substances," p. 55 (Japan Society for Promotion of Science, 1954).
3) M.E. Hale : Science, **123**, 671 (1956).

acid (λ_{max}^{EtOH} mμ (log ε) : 258 (4.10), 300~310 (3.5) (shoulder)) (Fig. 1) showed that it would be an orcinol type depsidone.

The evidences mentioned above account for the presence of one each of OH, OCH_3, COOH (chelated with *o*-hydroxyl), -CO-O- and -O- (IR_{max} ν : 1210 cm^{-1} (Nujol, KBr, $CHCl_3$)) in the molecule of grayanic acid.

Reduction of the depsidone skeleton, and the groups assigned as above from the molecular formula of grayanic acid, $C_{23}H_{26}O_7$, remains C_8H_{18}, which should be involved in the alkyl groups R and R′ in the following proposed structural formula (I).



I

A natural rule concerning with the nature of alkyl groups attached to the orcinol type lichen depsides and depsidones suggested that the alkyl groups, R and R′, of grayanic acid would be represented by those of odd carbon number and straight chain, which are shown in the following combination :

	R	R′	R+R′
1	CH_3	C_7H_{15}	C_8H_{18}
2	C_7H_{15}	CH_3	
3	C_3H_7	C_5H_{11}	
4	C_5H_{11}	C_3H_7	

The nuclear magnetic resonance (NMR) spectra of grayanic acid (in $CHCl_3$) (Fig. 2) indicated the presence of a methyl group attached directly to the aromatic benzene ring (τ=7.51 (signal (c)). Thus combinations (3) and (4) of R and R′ are ruled out.

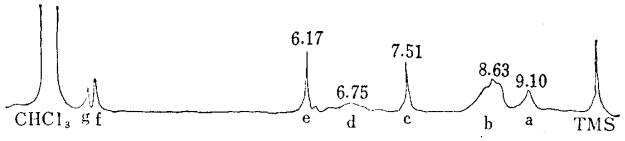


Fig. 2.

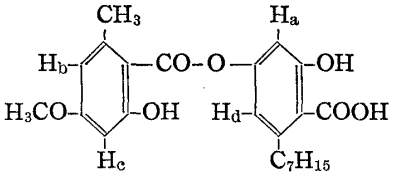
The signal (a) at τ=9.10 represents the terminal methyl of long alkyl chain and the broad signal (b) centered on τ=8.63 shows the intermediate methylenes. The signal (d) at τ=6.75 corresponds to the end methylene attached directly to the benzene ring.

The areal magnitudes of the characteristic signals which correspond to the number of protons were determined by weighing the paper cut out from the signals in NMR chart.

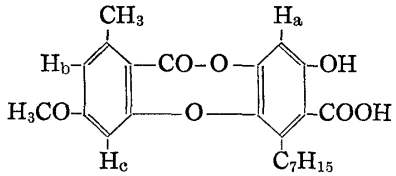
The result indicated that the weight of (a) + (b) is corresponding to 13 protons in comparison with that of (c) which represents 3 protons.

Thus the alkyl side chains, R and R′, of grayanic acid must be (1) CH_3 and C_7H_{15} or (2) C_7H_{15} and CH_3.

The chemical shifts (p.p.m. from TMS in acetone) of protons of benzene ring of grayanic acid were 6.13 (H_a), 6.66 (doublet) (H_c), and 6.80 (doublet) (H_b), which were compared with those of sphaerophorin 6.14 (H_a), 6.80 (H_b, H_d) and 6.43 (H_c).

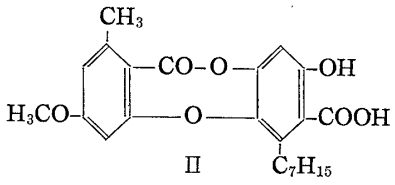


Sphaerophorin

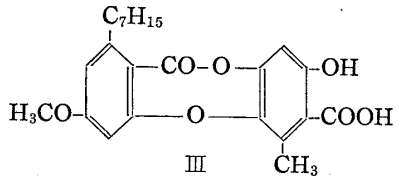


Grayanic acid

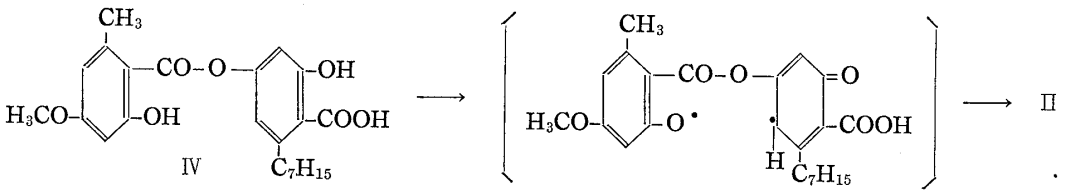
Of the alternative proposed structures of grayanic acid (II and III), the formula (II) seems to be more probable since it would be biogenetically corresponding to sphaerophorin (IV), a known depside which can be considered as being the precursor of grayanic acid.



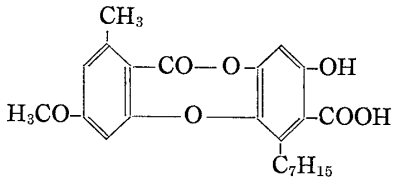
II



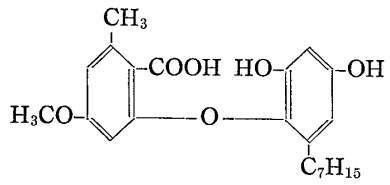
III



According to Jackman[4] the NMR signal of methyl of toluene (τ=7.66) is shifted to τ=7.15 by the introduction of $COOCH_3$ group in the *ortho*-position. This has been supported by the shift of methyl signal in orcinol(τ=7.75) and methyl orsellinate (τ=7.5).

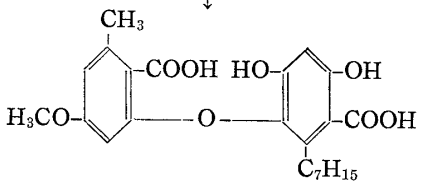


Grayanic acid (II)

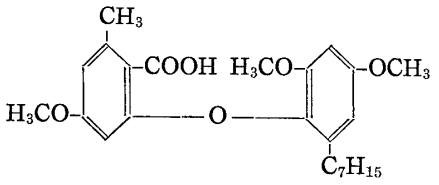


Grayanolic acid (VI)

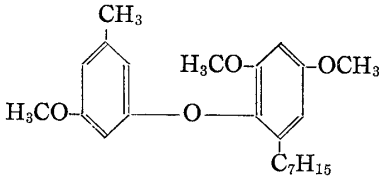
KOH, ice-cooling



grayanoldicarboxylic acid (V)

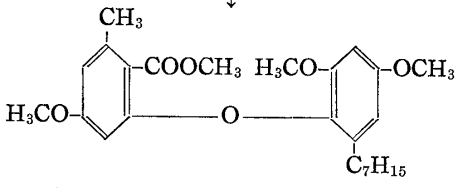


Grayanolic acid dimethyl ether (VII)



Grayanol dimethyl ether (VIII)

Cu chromite, quinoline



Methyl grayanolate dimethy ether (IX)

4) L. M. Jackman : "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," p. 58.

The NMR spectra of the degradation products of grayanic acid, grayanol dimethyl ether (Ⅷ), and methyl grayanolate dimethyl ether (Ⅸ), have been measured and the signal of methyl in the compound(Ⅷ, (τ=7.75) has been shown to shift to τ=7.65 in the compound (Ⅸ).

This indicates that methyl is located at the *ortho*-position of lactone linkage in the molecule of grayanic acid.

Consequently grayanic acid and its degradation products are formulated as illustrated below :

Experimental*2

Isolation of Grayanic Acid——*Cladonia Grayi* Merrill (50 g.) was extracted with Et_2O, and the extracts were recrystallized from 50% EtOH to obtain colorless needles, grayanic acid, m.p. 186~189°(decomp.). Yield : 350 mg. (0.7%). The unity of the crystals obtained was proved by thin layer chromatography. *Anal.* Calcd. for. $C_{23}H_{26}O_7$: C, 66.65; H, 6.32; CH_3O, 7.5. Found : C, 66.65, 66.91, 67.10; H, 6.39, 6.39, 6.39; CH_3O, 7.83 (Zeisel).

Grayanic acid gives a violet color with 1% $FeCl_3$ in ethanolic solution, and shows pale yellow color with diazonium reagent. It exhibits pale fluorescence under UV light illumination. A red coloration appeared on addition of 10% KOH and $CaOCl_2$ to grayanic acid turns immediately into yellow. It does not show a green fluorescence with KOH and $CHCl_3$, while it gives only red color (negative homofluorescein reaction). Grayanic acid is readily soluble in Et_2O, Me_2CO, EtOH, AcOEt, soluble in MeOH and $CHCl_3$, and sparingly soluble in benzene, and insoluble in hexane and petr. ether. It is soluble in aq. $NaHCO_3$ solution, and it forms an oily layer of Na salt between Et_2O and aqueous layer when the ethereal solution is shaken with aq. $NaHCO_3$ solution.

Acetate——Grayanic acid (50 mg.) dissolved in Et_2O (3 ml.) was mixed with Ac_2O (0.7 ml.) and, the mixture was allowed to stand for 3 days. An oily residue obtained after evaporation was recrystallized from benzene-hexane mixture to give colorless crystals, m.p. 157~159°(Yield : 34 mg.). *Anal.* Calcd. for $C_{25}H_{28}O_8$: C, 65.78; H, 6.18. Found : C, 65.88; H, 6.17. IR ν_{max}^{KBr} cm^{-1} : 1780 (phenolic acetate); 1695 (non bonded COOH).

Methyl Grayanate Methyl Ether——An excess of CH_2N_2 in Et_2O was added to the ethereal solution of grayanic acid (50 mg.), and the mixture was allowed to stand for 3 days. A few drops of MeOH were added to the mixture on the 3rd day to promote the reaction. The residue obtained on evaporation was recrystallized from aq. MeOH to obtain colorless needles, m.p. 88~90°. Yield : 40 mg. *Anal.* Calcd. for $C_{25}H_{30}O_7$: C, 67.85; H, 6.83. Found : C, 67.70, 68.24; H, 6.82, 6.84; mol. wt. (Rast.) 463.4. IR : ν_{max}^{Nujol} 1725 cm^{-1} (ester and lactonic linkage.)

Grayanol-dicarboxylic Acid (V)——Grayanic acid (25 mg.) was dissolved in 10% aq. KOH (0.5 ml.) and allowed to stand for 10 min. under ice-cooling. After acidification, the precipitates formed were recrystallized from a mixture of Me_2CO-benzene to form colorless crystals, m.p. 168~170° named grayanol-dicarboxylic acid (Yield : 16 mg.).

It gives a violet coloration with $FeCl_3$ and shows immediately with $CaOCl_2$ red turning into yellow. When the ethereal solution was shaken with aq. $NaHCO_3$ solution, it was taken completely into aqueous layer. *Anal.* Calcd. for $C_{23}H_{28}O_8$: C, 63.88; H, 6.53. Found : C, 64.02; H, 6.60. IR ν_{max}^{KBr} cm^{-1} : 1665 (COOH); 1635 (chelated COOH).

Grayanolic Acid (VI)——Grayanic acid (600 mg.) was added with $Ba(OH)_2$ solution (2.4 g. $Ba(OH)_2$ in 60 ml. H_2O), and the mixture was refluxed for 1 hr. The reaction mixture filtered and acidified with HCl, when an oily substance separated out, which was taken by shaking with Et_2O. On shaking the ethereal solution with aq. $NaHCO_3$ solution, it dissolved completely into the aqueous layer. IR : $\nu_{max}^{Cap.}$ 1700 cm^{-1} (nobonded COOH).

Dimethyl Ether (VII)——Grayanolic acid (450 mg.) was methylated on shaking with Me_2SO_4 (0.34 g.) and 5% NaOH (3 cc.) for 30 min. After addition of 5% NaOH (1.5 ml.) the mixture was boiled for 1 hr. On acidification, an oily substance separated out, which was taken with Et_2O and shaken with aq. $NaHCO_3$ solution to separate acid and ester. IR : $\nu_{max}^{Cap.}$ 1700 cm^{-1} (nobonded COOH).

Grayanol Dimethyl Ether (VIII) (Decarboxylation of Grayanolic Acid Dimethyl Ether)——The crude grayanolic acid dimethyl ether was dissolved into quinoline (1.4 ml.) and added with Cu chromite (67 mg.). The mixture was heated at 190°, and after CO_2 gas evolution ceased the reaction mixture was cooled and diluted with Et_2O. The ethereal solution was shaken first with dil. HCl to remove quinoline, and then with NaOH. The yellowish brown oily residue obtained by removing Et_2O was chomatographed

*2 All melting points were determined by Kofler's micro-method.

on alumina using benzene-hexane (1:1) as the developing solvent. The decarboxylated product, grayanol dimethyl ether (Ⅷ) was obtained in oil, which was not able to purify further due to its poor yield. NMR (in $CHCl_3$) $\tau=9.12$ (terminal Me of C_7H_{15}), 8.73 (intermediate methylenes of C_7H_{15}), 7.75 (aromatic Me), 6.26 (2 OCH_3), 6.17 (1 OCH_3).

Methyl Grayanolate Dimethyl Ether (IX)——Grayanolic acid dimethyl ether (Ⅶ) was methylated with CH_2N_2 in Et_2O. The brownish residue obtained on evaporation of the solvent was chromatographed on the silica gel column filled with a small amount of alumina on the top, using benzene as the developing solvent. The methyl ester was obtained as an oil. IR : $\nu_{max}^{CHCl_3}$ 1725 cm^{-1}(ester). NMR (in $CHCl_3$) $\tau=$ 9.15 (terminal Me of C_7H_{15}), 8.75 (intermediate methylenes of C_7H_{15}), 7.65 (aromatic Me), 6.35, 6.28, 6.20 (3 OMe), 6.11 ($COOCH_3$).

The authors wish to thank Professor Emeritus Y. Asahina for his kind advice and encouragement given us throughout this work and for the supply of lichen material.

The NMR spectra were determined by Dr. Kawazoe of this Faculty, Miss M. Onishi, Nihon Denshi Co., Ltd. and by the Members of Analytical Laboratory of the Research Laboratory of Mitsubishi-Kasei Co., Ltd., to whom the authors are very much indebted.

The measurements of IR and UV spectra, and the elemental analyses were carried out by the members of Microanalytical Laboratories of this Faculty, to whom the authors thanks are due. A part of expenses of the present study was supported by the grant provided by ministry of Educasion and Yakurikenkyu-kai to which the authors express their gratitude.

Summary

The structure of grayanic acid, $C_{23}H_{26}O_7$, m.p. 186~189° which was isolated from *Cladonia Grayi* MERRILL has been established to be an orcinol-type depsidone as formulated (Ⅱ).

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162. Yutaka Asahi : Polarography of Thioacetazone and its Related Compounds.*1,*2

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It has been reported by Dusinsky[1] that thioacetazone (I), an antituberculous chemical, shows a polarographic reduction wave in alkali and two reduction waves in acid, and it is assumed that the wave in alkali ascribes to 2-electron reduction of the azomethine group. The electrode process in acid, however, has not been revealed yet. Polarographic methods for the determination of thioacetazone in tablets have been reported by Mnoucek[2] and Icha.[3]

In this study, the polarographic determination of thioacetazone and its degradation products was an object at the early stage. The polarographic behaviors of thioacetazone and its related compounds such as benzaldehyde thiosemicarbazone (Ⅱ), S-methyl-

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*1 Polarographic Studies of Pharmaceuticals. XXVI. Part XXV. Y. Asahi : Bull. Chem. Soc. Japan, **34**, 1185 (1961).

*2 A part of this paper was read at the discussion meeting for polarography (Nov. 1956, in Tokyo).

*3 Higashiyodogawa-ku, Osaka (朝日 豊).

1) G. Dusinsky : Pharmazie, 8, 897 (1953).

2) K. Mnoucek, E. Knobloch : Czechoslov. farmacie, **2**, 306 (1953).

3) F. Icha : *Ibid.*, **2**, 308 (1953).