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STRUCTURE OF GRAYANOTOXIN-I AND -III*

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GRAYANOTOXIN-I (Andromedotoxin, Acetylandromedol) of Leucothoe grayana is one of the most widely-spread toxic substance in the plants of Ericaceae[1]. It has been shown by Takei et al.[2] that Grayanotoxin-I (G-I) has the molecular formula of $C_{22}H_{36}O_7$, and that alkaline hydrolysis gives acetic acid and Grayanotoxin-III (G-III), $C_{20}H_{34}O_6$, which is also a constituent of the Ericaceae plants[3]. We propose structure I (R=Ac) for G-I and I (R=H) for G-III, respectively, on the following evidence.

The refluxing of G-I, $C_{22}H_{36}O_7$, m.p. 272°, IR: 3540, 1738, 1380, 1250 cm^{-1} with anhydrous copper sulfate in

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[*] Presented at the 4th symposium on the natural organic products, Kyoto, Japan, October, 1960.

[1] T.Takemoto and H.Meguri, Japanese J. Pharm. Chem. **29**, 588 (1957).

[2] S.Takei and S.Miyazima, J. Agri. Chem. Japan **10**, 1093 (1934).

[3] H.Kakisawa and M.Kurono, Meeting of Chem. Soc. Japan, Kyoto, Japan, April, 1958.

acetone gave isopropylidene-G-I (II), $C_{25}H_{36}O_5$, m.p. 215°, IR: 3500, 1738, 1650, 1620 cm^{-1}, with loss of two moles of water[4]. Catalytic hydrogenation (2 mol. H_2) of this compound gave the saturated tetrahydro-isopropylidene-G-I (III), $C_{25}H_{40}O_5$, m.p. 163°, IR: 3500, 1736 cm^{-1}, which on chromic trioxide oxidation in pyridine yielded monoketo-tetrahydro-isopropylidene-G-I (IV), $C_{25}H_{38}O_5$, m.p. 186°, IR: 1750, 1740 cm^{-1}. The infrared spectra of IV suggested the presence of a five-membered ring ketone and the absence of hydroxyl group. Since the two double bonds of II are formed by dehydration of the two hydroxyl groups of G-I, it follows that G-I contains no ether linkage and that of the seven oxygen atoms contained in G-I ($C_{22}H_{36}O_7$) five of them should be hydroxyl groups, the remaining two being that of the acetoxyl group.

Three hydroxyl groups in G-IIIare evidenced to be tertiary because tribenzoyl-G-III (V)[5], $C_{41}H_{46}O_9$, m.p. 170°, is resistant to oxidation with chromic acid.

Both G-I and G-III consumed 1 mole of lead tetra-acetate or sodium metaperiodate to give G-I-ketoaldehyde (VI), $C_{22}H_{34}O_7$, m.p. 216°, IR: 3500, 1736 cm^{-1}, and G-III-ketoaldehyde (VII), $C_{20}H_{32}O_6$, m.p. 249°, IR: 3360, 1730 cm^{-1}, respectively. On the contrary the tribenzoate V and mono-

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[4] H.B.Wood, V.L. Stromberg, J.C.Kesesztesy and E.C.Horning, J. Am. Chem. Soc. **76** 5689 (1954).

[5] H.Meguri and T.Takemoto, J. Pharm. Soc. Japan **78**, 112 (1958).

acetyl-G-I[4] (VIII), $C_{24}H_{38}O_8$, m.p. 244°, were both stable to these reagents. Treating these keto-aldehydes, VI and VII, with alkali readily gave rise to the same unsaturated monoketone (IX), $C_{20}H_{30}O_5$, m.p. 248°, IR: 3430, 1720 cm^{-1}, which has been considered to be an internal aldol condensation product and free from an aldehydic function. From these transformations it is apparent that G-I (and G-III) has a cyclic α-glycol group, one hydroxyl group being tertiary and the other the secondary.

The presence of a secondary acetoxyl group which located in a five-membered ring was shown by oxidation of monoketo-isopropylidene-G-III (X), $C_{23}H_{36}O_4$, m.p. 198°, IR: 3440, 1735 cm^{-1}, obtained by alkaline hydrolysis of IV, to a diketone (XI), $C_{23}H_{34}O_4$, m.p. 200°. The IR spectrum of XI exhibited a single carbonyl peak at 1743 cm^{-1}. This conclusion was further supported by the following evidence. The Wolff-Kishner reduction product of the monoketone IV, a monoalcohol $C_{23}H_{38}O_3$, m.p. 132°, was oxidized to give a monoketone (XII), $C_{23}H_{36}O_3$, m.p. 129°, whose IR spectrum showed an absorption maximum at 1740 cm^{-1} due to a five-membered ring ketone.

Acetylation of isopropylidene-G-I (II) gave monoacetyl-isopropylidene-G-I (XIII), $C_{27}H_{38}O_6$, m.p. 138°, with following NMR[6] peaks: terminal methylene protons (2) at 62 cps.,

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[6] NMR spectra were measured with a Varian Associates instrument (40 Mc.) using 99.5 % deuteriochloroform as solvent and benzene as an external reference.

ethylenic proton (1) at 45 cps., methylprotons attached to ethylenic bond (3) at 181 cps., methylprotons of isopropylidene group (6) at 192 cps., methylprotons of acetoxyl (6) at 170 cps. and methylprotons (6) at 205 cps.

It has been shown cleary that terminal methylene and $H-C=C-CH_3$ system are present in XIII, since ozonisation of keto-isopropylidene-G-I (XIV), $C_{25}H_{34}O_5$, m.p. 184°, IR: 1732, 1745 cm^{-1}, which was obtained by the oxidation of II, produced an appreciable amount of formaldehyde and nortriketo acid, $C_{24}H_{32}O_9$, m.p. 298°, IR: 3500-3000, 1755, 1705 cm^{-1}.

From these data, especially the results of NMR, there should be four methyl groups in G-III; however, the Kuhn-Roth C-methyl determination indicated only three such groups to be present. This difference coupled with a doublet at ca. 1370 cm^{-1} in the IR spectra of all derivatives of G-I suggest the presence of a <u>gem</u>-dimethyl group.

Fig. 1 shows the functional groups of G-I thus obtained.

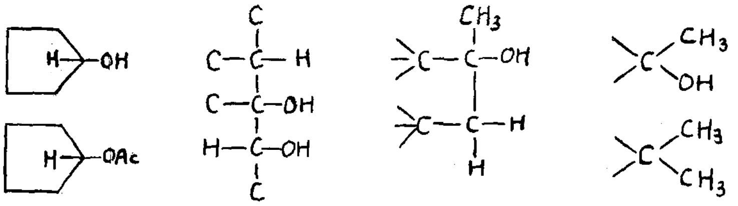

Fig. 1

Oxidation of G-I-ketoaldehyde VI with bromine followed by alkaline hydrolysis gave an α,β-unsaturated acid (XV), $C_{20}H_{30}O_6$, m.p. 215°, methyl ester (XVI), $C_{21}H_{32}O_6$, m.p. 177°.

Ultraviolet absorption of XV at 256 mμ (ε10960) indicated the existence of an α,β-unsaturated ketonic function, which was further shown to be a s-cis-α,β-unsaturated five-membered ring ketone moiety because the 1600 cm^{-1} IR peak intensity was almost comparable to that of the 1705 cm^{-1} carbonyl peak[7].

Acid treatment of acid XV or methyl ester XVI readily gave a lactone (XVII), $C_{20}H_{28}O_5$, m.p. 225°, λ_{max}^{EtOH} 253 mμ (ε11200), IR: 3500, 1782, 1705, 1600 cm^{-1}, therefore; a hydroxyl group should be in a γ-position to the carboxyl group in XV. The lactone XV was oxidized with chromic acid anhydride in acetic acid to gave a yellow lactone (XVIII), $C_{20}H_{26}O_6$, m.p. 236°, λ_{max}^{EtOH} 235 (10600), 320 mμ (ε20), IR: 3480, 1780, 1745, 1705 cm^{-1}, which was reduced and discolored by Zn-AcOH. The chemical and spectral characteristics of XVIII were quite similar to cyclopentene-3,5-dione (XIX)[8]. Furthermore, since the infrared and ultraviolet absorptions characteristic of the s-cis-α,β-unsaturated ketone moiety disappeared in XVII, it is apparent that double bond in XV migrates to form an enedione system in XVI.

The foregoing results lead to the partial structure XX for G-I (R or R'=Ac) and reactions are summarized in Fig. 2 on the structure XX.

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[7] E.S.Waight and R.L.Erskine, Steric Effects in Conjugated Systems, p. 73, Butterworths publications Ltd., 1958.

[8] C.H.DePuy and E.F.Zaweski, J. Am. Chem. Soc. 81, 4920 (1959).

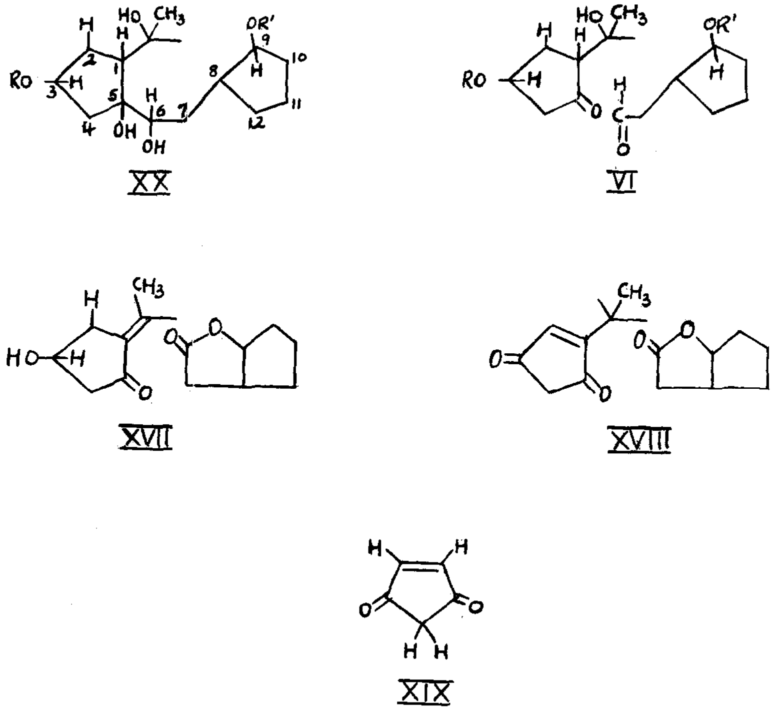
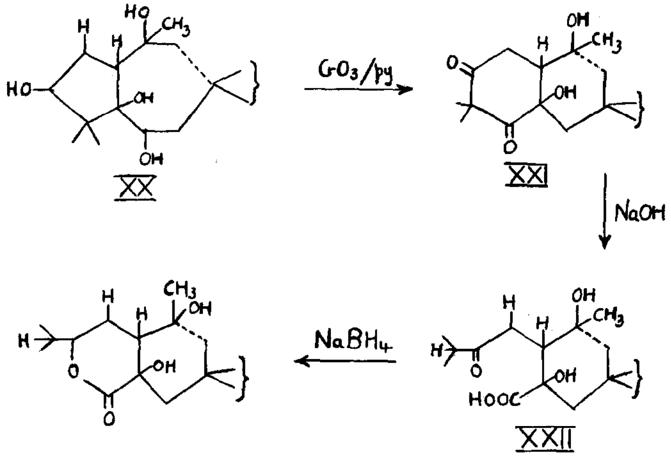


Fig. 2

Further evidence for XX, and confirmation of the position of the acetoxyl group was acquired through the following reaction: oxidation of G-I with chromic trioxide in pyridine yielded diketo-G-I, (XXI) $C_{22}H_{32}O_7$, m.p. 248°, IR: 3500, 1735, 1713 cm^{-1}; alkaline hydrolysis of this gave the monoketo-acid (XXII), $C_{20}H_{32}O_7$, m.p. 213°, IR: 3450, 1708 cm^{-1}, methyl ester (XXIII), $C_{21}H_{34}O_7$, m.p. 198°. This acid XXII yielded a δ-lactone, $C_{20}H_{32}O_6$, IR: 3480, 1730 cm^{-1}, upon reduction with $NaBH_4$, and a diketo-acid, $C_{20}H_{30}O_7$, m.p. 248°, IR: 3460, 1742, 1700 cm^{-1}, upon oxidation with chromic trioxide in acetic acid, respectively. These transformations are interpreted as follows: the C_9-hydroxyl group in the partial structure XX of G-I is originally acetylated and the remaining two secondary hydroxyl groups are oxidized with simultaneous acyloin migration[9] to a β-diketone XXI and these transformed as indicated below.



Hydrolysis of monoketo-isopropylidene-G-I (XIV), followed by oxidation with chromic trioxide in pyridine afforded

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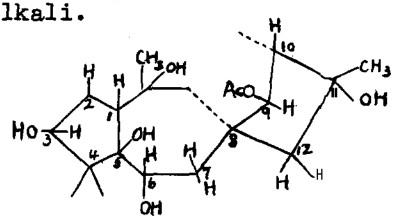
[9] D.G.Hardy, W.Rigby and D.P.Moody, J. Chem. Soc. 2955 (1957). W. Cocker, J.T.Edward, T.F.Holley and D.M.S. Wheeler, Chem. Ind. 1485 (1955).

diketo-isopropylidene-G-III (XXIV), $C_{23}H_{30}O_4$, m.p. 213°, IR: 1740, 1748, 1660, 1625 cm^{-1}. Its NMR spectra, besides peaks assigned due to terminal methylene (30 cps.), $H-C=C-CH_3$ (50 cps. and 178 cps.), isopropylidene-dimethyl (197 cps.) and gem-dimethyl (207 cps.), showed a quartet at 80 cps. arising from the C_6-hydrogen in partial structure XX; thus C_7 should have two hydrogen atoms.

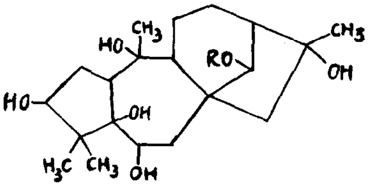
Evidence has been obtained which suggests a $H-C=C-CH_3$ system occupying a position β to C_9 in partial structure XX. Namely, monoketo-isopropylidene-G-I (XIV) was oxidized by OsO_4 and alkaline hydrolysis gave a mixture of pentol and triol. Further oxidation of this mixture with sodium metaperiodate yielded triketo-aldehyde, $C_{22}H_{30}O_7$, m.p. 234°, the mother liquor of which furnished another compound, α,β-unsaturated keto-aldehyde, $C_{23}H_{26}O_5$, m.p. 235°, λ_{max}^{EtOH} 232 mμ (ε13300), IR: 2880, 1760, 1715, 1670 cm^{-1}, when chromatographed on alumina.

From these results it is possible to extend the partial structure XX for G-I to XXV. C_{10}-hydrogen in XXV must be in a bridge-head position because the diketone XXIV was not converted to an α,β-unsaturated ketone when treated with alkali.



XXV

Selenium dehydrogenation of G-III yielded a very small amount of aromatic hydrocarbon which was characterized as trinitrobenzene adduct m.p. 141°, λ_{max}^{EtOH} 252, 260, 330, 340, 346, 366, 390 mμ; since the ultraviolet spectrum is very similar to that of anthracene, it is quite reasonable to ascribe an alkyl anthracene structure for the dehydrogenation product. Since G-I is a tetracyclic and since two rings are present as five-membered rings, the formation of an alkylanthracene from G-III should require a rearrangement during the course of dehydrogenation and also the presence of a seven-membered ring adjacent to one of the five-membered rings. These facts coupled together with biogenetic considerations lead to structure I (R=Ac) and I (R=H) for Grayanotoxin-I and -III, respectively.



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Several data not given in this communication also support these structures.

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