Tetrahedron 1965, Vol. 21, pp. 3091 to 3104. Pergamon Press Ltd. Printed in Northern Ireland

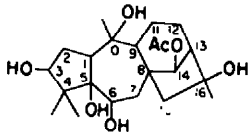
STEREOCHEMISTRY OF GRAYANOTOXINS

H. KAKISAWA,* T. KOZIMA,† M. YANAI* and K. NAKANISHI†

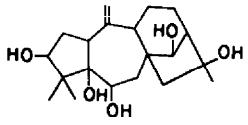
(*Received* 12 *April* 1965; *in revised form* 24 *May* 1965)

Abstract—The stereochemistry of the grayanotoxins is discussed on the basis of chemical and physicochemical data, and the stereostructures XLI, XLII and XLIII have been assigned to grayanotoxin-I, -II and -III, respectively.

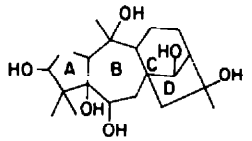
THE toxic constituents, Grayanotoxin-I, Grayanotoxin-II and Grayanotoxin-III, of the leaves of the various *Ericaceae* species have been the subject of numerous investigations for the last half century, and recently their structures have been elucidated as I, II and III, respectively.[1,2,3] The present paper describes in detail the stereochemistry of these three substances, which has been partly presented as preliminary communications.[4,5]



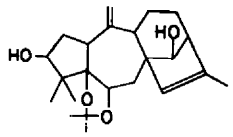
Grayanotoxin – I



Grayanotoxin – II



Grayanotoxin – III



IV

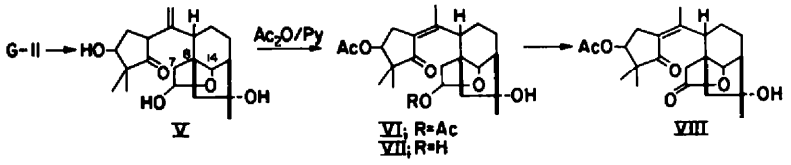
Alkaline hydrolysis of Grayanotoxin-I (abbreviated as G-I) afforded Grayanotoxin-III (G-III), which could be dehydrated under mild condition to yield Grayanotoxin-II (G-II).[6] Furthermore, treatment of G-II or G-III with anhydrous copper sulphate and acetone afforded the same isopropylidene derivative IV.[6] It follows that the asymmetric centers of the three grayanotoxins all belong to the same series, respectively.

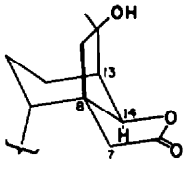
The stereochemistry of the C- *and* D-*rings.* Oxidative cleavage of the α-glycol bond of G-II with sodium periodate gave dehydro-G-II. Since no aldehyde absorption appeared in the IR spectrum of this product, apparently a five-membered hemiacetal V had been formed. Acetylation of this dehydro-G-II with pyridine and acetic

* Department of Chemistry, Tokyo Kyoiku University, Tokyo.
† Department of Chemistry, Tohoku University, Sendai, Japan.

[1] H. Kakisawa, M. Kurono, S. Takahashi and Y. Hirata, *Tetrahedron Letters*, No. 2, 59 (1961).
[2] H. Kakisawa, *J. Chem. Soc. Japan* **82**, 1096, 1216 (1961).
[3] J. Iwasa, Z. Kumazawa and M. Nakajima, *Chem. & Ind.* 511 (1961); *Agr. Biol. Chem.* **25**, 782 (1961).
[4] H. Kakisawa, M. Yanai, T. Kozima, K. Nakanishi and H. Mishima, *Tetrahedron Letters* 215 (1962).
[5] T. Kozima, K. Nakanishi, M. Yanai and H. Kakisawa, *Tetrahedron Letters* 1329 (1964).
[6] H. Meguri, *J. Pharm. Soc. Japan* **79**, 1060 (1959).

anhydride was accompanied by a shift of the double bond to give the diacetyl α,β-unsaturated carbonyl compound VI, λ_{max} 255 mμ (ε 16,300),[16a] which was hydrolysed with hydrochloric acid to give a monoacetyl α,β-unsaturated compound VII. Chromic trioxide oxidation of the monoacetate (VII) afforded the keto-γ-lactone (VIII) the IR spectrum of which showed a lactone band at 1770 cm^{-1}, and α,β-unsaturated ketone bands at 1710 and 1615 cm^{-1}. Since the five-membered hemiacetal or lactone rings are fused to the five-membered D-ring in derivatives V to VIII and since these derivatives were formed under mild conditions, the two five-membered rings should be *cis*-fused (IX).[7] Namely, the C_{14}–OR and the C_7–C_8 linkages in G–I, G–II and G–III are in a *cis* relation with respect to D-ring.



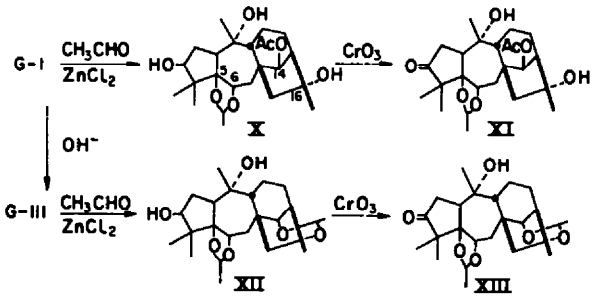


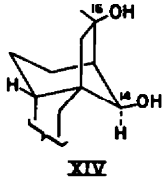
IX

An additional support for the configuration of C_{14}–OR is provided by the NMR spectrum of the lactone (VIII), which showed a quartet at δ 4·79 ppm (J_{AX} 3·8, J_{BX} 5·5 c/s) and a singlet at δ 4·57 ppm, each corresponding to one proton; from comparison with other NMR spectra, the former is assigned to the C_3–H and the latter to the C_{14}–H. Dreiding models indicate that the dihedral angle between the C_{14}–H and the C_{13}–H is nearly 90° when the C_{14}–H has the axial α-configuration as shown in the partial structure IX, and about 40° when the C_{14}–H has equatorial β-configuration. Thus the C_{14}–H signal would be expected to appear as a singlet and doublet, respectively in the two configurations. The C_{14}–H is accordingly oriented axial (α).

G-I reacts with acetaldehyde and zinc chloride to yield the ethylidene derivative (X), that could be oxidized to a five-membered ketone (XI), the IR spectrum of which showed a ketone band at 1739 cm^{-1} and an acetate band at 1729 cm^{-1}. The ethylidene group therefore must be attached to the α-glycol moiety. Similar treatment of G-III afforded an amorphous diethylidene derivative (XII) that could also be oxidized to a crystalline five-membered ketone (XIII) having an IR band at 1747 cm^{-1}. Elementary analysis of the latter compound (XIII) showed that two molecules of acetaldehyde had been introduced. Since one molecule of acetaldehyde forms an acetal ring between C_5–OH and C_6–OH, it is only between C_{14}–OH and C_{16}–OH that another molecule of acetaldehyde can produce a similar ring. The structure of XIII is also supported by its NMR spectrum which shows two quartets at 4·94 (J 5 c/s) and 5·16 (J 5 c/s) corresponding to the two acetal methine protons. The C_{14}–H signal still appears as a singlet at 4·51 ppm while the C_6–H signal appears as a broad

[7] G. Stork and F. K. Clark, *J. Amer. Chem. Soc.* **83**, 3114 (1961).



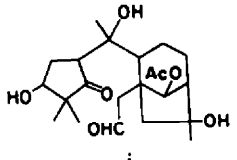


XIV

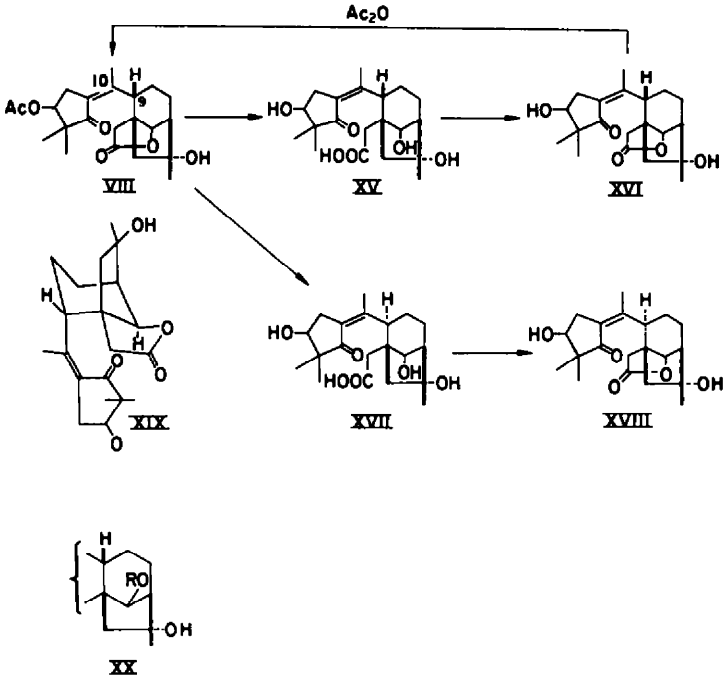
absorption at 4·26 ppm with a half-band width of 8 c/s. The formation of these acetal rings establishes the *cis*-relation of C_{14}–OH and C_{16}–OH with respect to ring-D (XIV).

The stereochemistry of B/C *ring junction and of* C_{10}–OH. As described above G-II was converted to the monoacetyl-α,β-unsaturated ketolactone (VIII). Mild alkaline hydrolysis of this lactone afforded the corresponding carboxylic acid (XV)[8]: ν 3400, 1715, 1695 and 1600 cm^{-1}, λ_{max} 258 mμ (ε 10,960), which when heated with acetic acid was easily converted into the γ-lactone (XVI)[2] ν^{KBr} 3500, 1782, 1700 and 1600 cm^{-1}; λ_{max}^{EtOH} 256 mμ (ε 11,200). Acetylation of the lactone XVI gave back the original compound (VIII), and accordingly the conversions involve no change in the stereochemistry of the asymmetric centres. On the other hand, alkaline hydrolysis of the monoacetyl-γ-lactone (VIII) under more vigorous conditions gave an isomeric carboxylic acid (XVII), ν 3370, 1715, 1700 and 1598 cm^{-1}; λ_{max} 260 mμ (ε 12,000), which when heated with acetic acid yielded the corresponding γ-lactone (XVIII), ν 3420, 1780, 1702 and 1605 cm^{-1}; λ_{max} 255 mμ (ε 14,600). Spectroscopic properties of the two carboxylic acids (XV and XVII), and those of the two γ-lactones (XVI and XVIII), were respectively quite similar, excepting minor differences in the finger print region of the IR spectra. Hence it is apparent that these compounds represent two series of stereoisomers and that they are epimeric at C_9, which is conjugated to the C_5-carbonyl through the double bond and can be epimerized by base. Thus, the

[8] The same acid was also obtained from G-I. Namely, oxidative cleavage of the α-glycol bond of G-I with sodium periodate gave a keto-aldehyde i, which was further oxidized with Br_2 and then subjected to mild alkaline hydrolysis to afford XV.



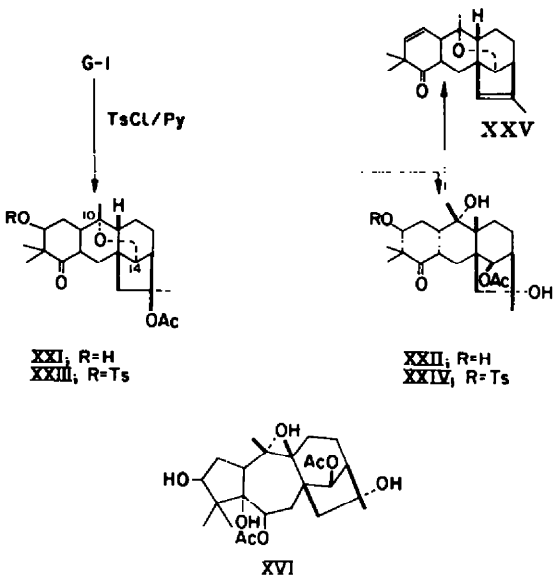
i



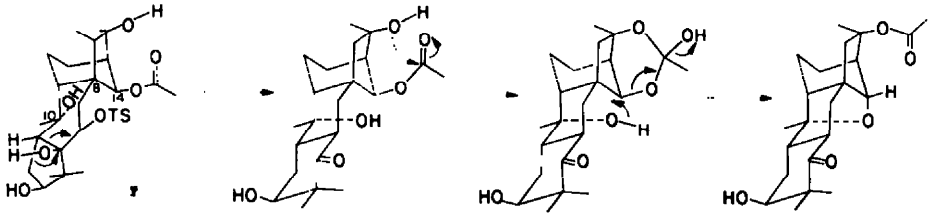
C_9–C_{10} bond in the γ-lactone (VIII) should be axial with respect to ring C, and this is epimerized to the more stable equatorial conformation in carboxylic acid (XVII). These data indicate that the γ-lactone (VIII) can be represented by the conformational structure XIX and that the B/C ring junctions of grayanotoxins are *cis*, i.e. XX or its mirror image.

The *cis* relationship of the B/C ring junction of the grayanotoxins is further confirmed and the configuration at C_{10}–OH is also settled from the following observations. Treatment of G-I with one mole of *p*-toluenesulphonyl chloride and pyridine for 2-3 weeks at room temperature or 1 hr at 100° gave two products, the monoacetate monoalcohol (XXI) and the monoacetate diol (XXII). Since their IR spectra indicate the formation of a six-membered ketone (ν^{CHCl_3} 1700 and 1710 cm^{-1}, respectively), the five-seven-membered A/B ring system has evidently changed into a six-six-membered ring by a pinacol type rearrangement of the α-glycol group of G-I. The NMR spectrum of XXII shows five singlets at δ 1·10, 1·17, 1·30, 1·37 and 2·15 ppm corresponding to five methyl groups; appearance of the C_{14}–H signal as a singlet at δ 6·22 ppm proves that the environments of C_{14} remain unchanged (as in compound VIII) although the ca. 0·5 ppm downfield shift is to be noted.[9] On the other hand, in the product XXI there is a one-proton doublet at δ 4·42 ppm (J 5 c/s), and this indicates a structural change has occurred around C_{14}. With two moles of *p*-toluenesulphonyl chloride and pyridine G–I gave the ketotosylates (XXIII and XXIV), which in turn can also be prepared by tosylations of XXI and XXII, respectively. Similar to the monoacetate-triol (XXII), the NMR spectrum of monotosylate (XXIV) also exhibited a singlet at δ 6·18 ppm arising from the C_{14}–H, whereas the NMR spectrum of monotosylate (XXIII) again had a doublet at 4·43 ppm (J 5 c/s)

[9] This down-field shift is attributed to the shorter distance between the C_{14}–H and C_{10}–OH in XXII.



due to C_{14}–H. The fact that the α-glycol group is responsible for these rearrangements and that the tosyl group in XXIII and XXIV are introduced after the rearrangement is supported by the recovery of diacetyl G–III[1,2] (XXVI) without even being tosylated at C–3 under more vigorous conditions. Elementary compositions of XXI and XXIII indicated that they contained one molecule of water less as compared to XXII and XXIV, respectively. Neither olefinic nor vinyl methyl signals were present in the NMR spectra of XXI and XXIII, and furthermore, it is a secondary hydroxyl in XXI that is tosylated to give XXIII (HO—C—H at δ 3·58 is shifted to 4·44 ppm in TsO—C—H). Also, XXI and XXIII still retain the acetyl group (from IR and NMR spectra). Thus an ether linkage has been formed in these compounds, and the only structure for such an ether which is compatible with the mentioned findings is that involving formation of a C_{10}– to C_{14}–oxygen bridge and a concerted 1,3-acetyl migration. The rearrangement of G–I to the ether XXI is shown in the Scheme. Thus the



XXI

C_{10}–OH bond should be oriented towards the same direction as the C_8–C_{14} bond with respect to ring-B. This single tosylation also establishes the stereochemistry of all asymmetric centres on rings C and D, and especially the B/C ring junctions. The fact that shapes of the C_{14}–H NMR signals change from a singlet in the compounds XXII and XXIV to a doublet (J 5 c/s) in the ethers XXI and XXIII is also accounted for by these structures, because in the former two compounds the dihedral angle between

C_{13}–H and C_{14}–H is roughly 90°, whereas in the latter two it is approximately 40°; these relations between the J constants and dihedral angles are what would be expected from the Karplus equations.[10,11] An anhydrous compound XXV also having the C_{10}–O–C_{14} linkage was obtained by treatment of XXIV with collidine; the structure of this compound was assigned from the IR and UV spectra and elementary analysis (Experimental).

It has been reported that isopropylidene G–I (IV) can be transformed into the monoketone (XXVII)[1,2] (Fig. 1) by catalytic hydrogenation of the double bonds,

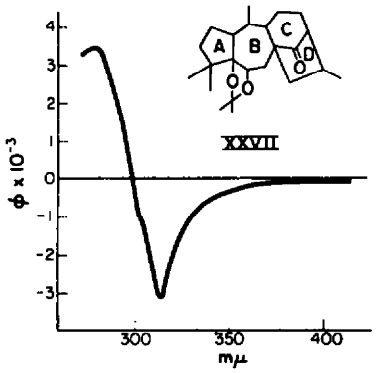
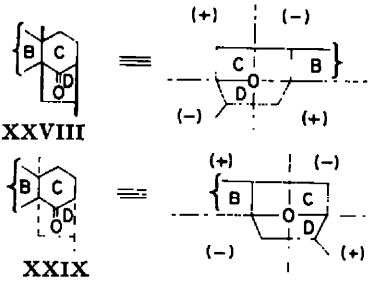


FIG. 1

removal of C_3–OH by chromic trioxide oxidation and Wolff-Kishner reduction, and subsequent chromic trioxide oxidation. The ORD curve of the monoketone (XXVII) showed a conspicuous negative Cotton effect (Fig. 1). Since the C_{14}-ketone is symmetrically placed on rings C and D, the sign of Cotton effect will be determined by ring B and the C_{16}-methyl group, both of which come into diagonally located octants (XXVIII and XXIX); furthermore, conformations of rings C and D are rigidly fixed,

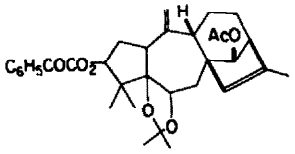


and consequently this ketone becomes particularly suited for deducing the absolute configuration of the C/D ring system. The negative Cotton sign of monoketone XXVII indicates that the absolute configuration should be XXVIII and not its mirror image XXIX.

The configurations at C_6 *and* C_3 *and the stereochemistry of the* A/B *ring junction.* It was first attempted to apply Prelog's asymmetric synthesis[12] to determine the

[10] M. Karplus, *J. Chem. Phys.* **30**, 11 (1953); *J. Amer. Chem. Soc.* **85**, 2870 (1963).
[11] K. L. Williamson and W. S. Johnson, *J. Amer. Chem. Soc.* **83**, 4623 (1961).
[12] V. Prelog, *Helv. Chim. Acta* **36**, 308, 320, 325 (1953).

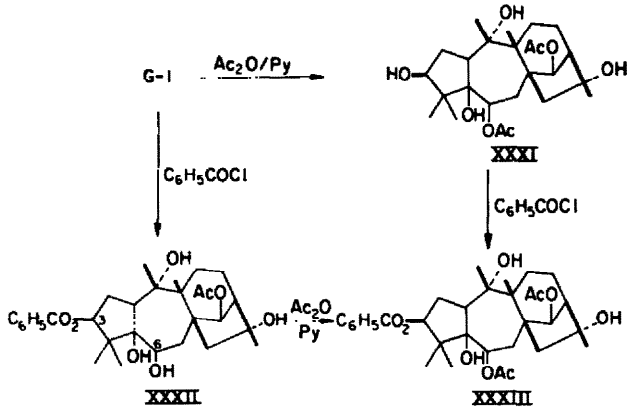
configuration of the C_3–OH group. Although the phenylglyoxylate (XXX) of the isopropylidene derivative could not be obtained in a crystalline state after repeated trials, the purity was checked by chromatography and the structure was supported by the IR and the UV spectra (Experimental). The asymmetric synthesis with the ester (XXX), however, gave only the optically inactive atrolactic acid in 40% esterification yield. Presumably the asymmetric synthesis had failed because of the presence of other nearby O-functions.[13] Several phenylglyoxylates were obtained as crystals but



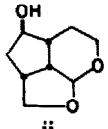
XXX

they were not employed for deducing the configuration in view of their anomalous spectroscopic properties; namely, heights of the λ_{max} of these esters were reduced to one half (ε 5000–7000) in methanol as compared to the values in other solvents, e.g. dioxan.[14] The absolute configuration of the C_3–hydroxyl was accordingly determined by the benzoate rule[15] and Horeau's asymmetric synthesis.[16]

The reactivities of the C_3–OH and C_6–OH are somewhat anomalous. Namely, reaction of G–I with acetic anhydride and pyridine resulted in acetylation at C_6 to give the acetate (XXXI), whereas reaction with benzoyl chloride resulted in benzoylation at C_3 to give the benzoate XXXII.[16a] The benzoate consumed one mole of



[13] A similar 0% optical yield has been experienced with tetrahydroanhydroaucubigenin (ii); A. Karube, M.Sc. Thesis, Tohoku University, Japan.

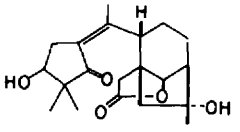


ii

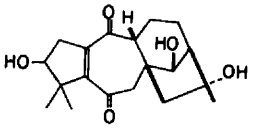
[14] T. Kozima, *Chem. Pharm. Bull.* in press.
[15] J. H. Brewster, *Tetrahedron* **13**, 106 (1961).
[16] A. Horeau and H. B. Kagan, *Tetrahedron* **20**, 2431 (1964).
[16a] T. Kozima, Ph.D. Thesis, Tohoku University, Sendai, Japan (1964).

periodate, and when acetylated with acetic anhydride and pyridine gave the triester (XXXIII; amorphous) which could also be prepared by benzoylation of the diacetate (XXXI). The carbinol G-I (1) and its 3-benzoate (XXXII) are a suitable pair for application of the benzoate rule, which predicts the benzoate to be more dextrorotatory if the C_3–OH had the β-configuration (S configuration) and vice versa. Actually this was found to be the case: benzoate (XXXII) $[\phi]_D$ +70·0° vs. G-I $[\phi]_D$ 0° (in dioxan); it follows that the configuration of the C_3–OH is β.

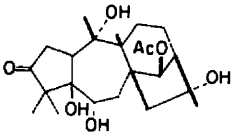
This conclusion is corroborated by the asymmetric syntheses employing α-phenylbutyric anhydride;[16] in addition, the configuration at C_6–OH was also deduced by this method. Compounds XVI[2] and XXXIV[3] were employed for determining the C_3-configuration while compounds XXXV[2] and G-I (1) were employed for deter-



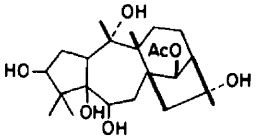
XVI



XXXIV



XXXV



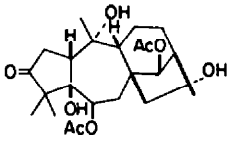
I

mining the C_6-configuration. Although XXXIV and I contain two secondary hydroxyl groups, the C_{14}–OH in XXXIV and C_3–OH in I are not acetylated by the usual treatment with acetic anhydride and pyridine, and accordingly they can be safely used for determination of configurations at C_3–OH and C_6–OH, respectively. From the rotations of the α-phenylbutyric acids liberated from XVI and XXXIV (negative) and from XXXV and I (positive) (Experimental), the configurations of the C_3- and C_6-hydroxyl groups are deduced to be both β. The very high optical yield (92%) in the case of compound XVI indicates that there is a substantial difference in the bulkiness at C_2 and C_4.

The stereochemistry of A/B ring junctions of grayanotoxins is determined from the ORD curve of 3-keto-derivatives XIII, XXXVI[2] and XXXVII.[2] The amplitudes[17] of these compounds are shown below the structures. A/B trans 2-keto-A-norsteroids,[18,19] their 4,4-dimethyl derivatives[20,21] and 16-ketosteroids,[19,21] all of which have the trans hexahydroindan-2-one moiety, exhibit very strong Cotton effects (a: 220–280). The cyclopentanone ring in these compounds are all fixed in the half chair form (or twist form), and Klyne[22] has pointed out that this twist greatly enhances the unsymmetric

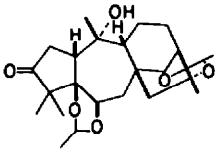
[17] C. Djerassi and W. Klyne, *J. Chem. Soc.* 4929 (1962).
[18] Jean-Francois Biellman and Mladen Rajic, *Bull. Soc. Chim. Fr.* 441 (1962).
[19] C. Djerassi, R. Riniker and B. Riniker, *J. Amer. Chem. Soc.* **78**, 6362 (1956).
[20] R. Hanna, C. Sandris and G. Ourisson, *Bull. Soc. Chim. Fr.* 1454 (1959).
[21] R. Hanna and G. Ourisson, *Bull. Soc. Chim. Fr.* 1945 (1961).
[22] W. Klyne, *Tetrahedron* **13**, 29 (1961).

nature of the ring, which consequently results in the large amplitude of the Cotton effect. On the contrary, the A/B *cis* 2-keto-A-norsteroid system exhibits a Cotton effect of greatly reduced amplitude (a: −155).[19] Not much data is available regarding the ORD properties of the 5/7-membered ring system such as that present in the grayanotoxins. However, a *trans* configuration has been assigned to the ring junction



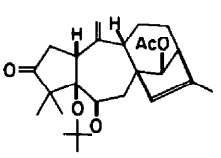
a: − 62 (CH_3OH)

XXXVI



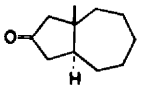
a: − 107 (CH_3OH)

XIII



a: − 119 (dioxane)

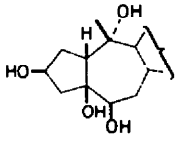
XXXVII



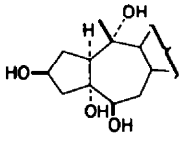
a: + 156

XXXVIII

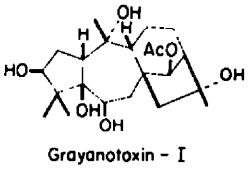
of a guanolide on the basis of its strong positive Cotton effect,[23] while Djerassi and Gurst[24] have shown that the sign and amplitude of the ORD curve of the *trans* 5/7-membered ring system such as XXXVIII do not differ greatly from those of the *trans* 5/6-system. This implies that the twist conformation of cyclopentanone is not altered to a great extent when the six-membered ring of 2-ketohydroindane is replaced by a seven-membered ring. On the other hand, the 3-keto derivatives (XIII, XXXVI and XXXVII)[2] all exhibit weak Cotton effect curves (especially XXXVI), which indicate a *cis* A/B ring junction. Thus two alternative possibilities, XXXIX and XL, come into consideration by taking into account the β-configuration of the C_6-hydroxyl as derived above. Of these two, the latter can be eliminated on the basis of the following evidence. Firstly, inspection of Dreiding models reveals that the two hydroxyl groups are rigidly fixed in an *anti-trans* arrangement in XL and this is against the facile formation of isopropylidene and ethylidene derivatives; the easy cleavage of the α-glycol moiety by periodate can also not be explained. Secondly, a



XXXIX

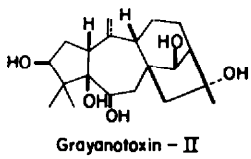


XL



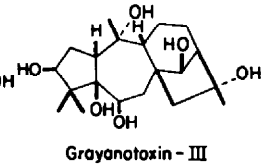
Grayanotoxin − I

XLI



Grayanotoxin − II

XLII



Grayanotoxin − III

XLIII

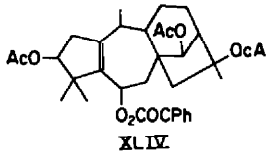
[23] F. Sanchez-Viesca and R. Roma, *Tetrahedron* **19**, 1285 (1963).

[24] C. Djerassi and J. E. Gurst, *J. Amer. Chem. Soc.* **86**, 1755 (1964).

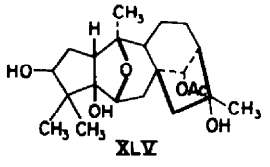
positive sign is predicted[22] for the ORD Cotton effect of the 3-keto compound XXXVI having the partial structure XL, which is against facts.

The full stereochemistry of the three grayanotoxins, I, II and III can thus be represented by structures XLI, XLII and XLIII, respectively.

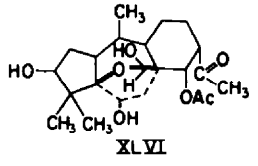
Kumazawa *et al.*[25] have assigned α-configurations to C_1–H and C_5–OH, but the stereochemistry deduced for the other centres is in agreement with results given here. Their evidence for a C_1–α–H configuration is based on $[M]_D$ values of two diketo lactones epimeric at C_1 (formula XVI with C_3–oxo and C_{10}–C_1 double bond saturated); namely, the $[M]_D$ values were calculated according to Brewster's "Conformational dissymmetry rule"[26] and these were compared with the observed values. However, such a structure is too complicated for straightforward application of this empirical rule, and in our view does not provide conclusive evidence for a C_1–α–H configuration. The configuration of the C_6–OH was then deduced by application of Prelog's asymmetric synthesis to compound XLIV, which gave (+)-atrolactic acid in an optical yield of 8% (corresponding to an α-configuration). The C_5–OH configuration was then assumed to be α since Dreiding models with the C_1–H and C^6–OH both in the α-configurations indicated that no isopropylidene derivative could be formed between C_5 and C_6. However, it is not clear which side of the centre at C_6 should be regarded as the larger group and accordingly, the C_6–α configuration is again not clear.



XLIV



XLV



XLVI

After publication of our preliminary communications[4,5] Tallent also assigned the same stereochemistry to all centers in acetyl andromedol (grayanotoxin-I),[27] excepting that at C_3 which was left undefined. His argument is based on the formation of the ether (XLV) and the hemiacetal (XLVI). Curiously enough, it is not possible to construct molecular models of a compound having the structure XLV. Furthermore, his evidence for the hemiacetal formation between a C_{15}-aldehyde function and C_5–OH is solely based on NMR chemical shifts of the C_4-gemdimethyl group, which could have been applied similarly to a hemiacetal between a C_{15}-aldehyde and C_6–OH. In view of the fact that the mere formation of isopropylidene or ethylidene derivatives do not nessarily indicate a *cis* relation between the C_5 and C_6 hydroxyls (because of the flexibility of ring B), the hemiacetal formation can only be used for deducing the configuration at one centre, i.e., either C_5 or C_6, and only after the ring-size has been established.

[25] IUPAC Symposium on the *Chemistry of Natural Products, Abstracts* p. 43. Kyoto (1964).
[26] J. H. Brewster, *J. Amer. Chem. Soc.* **81**, 688 (1954).
[27] W. H. Tallent, *J. Org. Chem.* **27**, 2968 (1962); **29**, 2756 (1964).

EXPERIMENTAL

The NMR spectra were measured with a Varian A-60 model (TMS internal reference) and the ORD curves with a self-recording JASCO ORD/UV-5 model.

α,β-Unsaturated ketolactol acetate (VII). G-II (II; 6 g) was dissolved in MeOH (180 ml) and water was added in a quantity (200 ml) just short of the precipitation point. This solution was mixed with an aqueous solution (35 ml) of sodium periodate (5·5 g) and allowed to stand overnight at room temp. The aqueous solution was then extracted with ethyl acetate (1·3 l.). The combined ethyl acetate extracts were washed with water (1 l.) and concentrated under red. press. to give dehydro G-II (V; 4·5 g). This compound (V) was allowed to stand overnight at room temp with acetic anhydride (15 ml) and pyridine (38 ml). The reaction mixture on being poured into ice water yielded an oily substance which, after acidification followed by heating with stirring, afforded crystals with dissolution of the oil. Recrystallization from MeOH–water gave VII as colourless fine needles (3·3 g), m.p. 193–195°. IR ν_{max}^{KBr} cm^{-1}: 3375, 3220, 1744, 1713, 1609. (Found: C, 67·65; H, 8·14. $C_{23}H_{32}O_6$ requires: C, 67·32; H, 8·22%.)

α,β-Unsaturated ketolactone acetate VIII. The above acetal (VII; 0·75 g) was dissolved in acetic acid (15 ml) and allowed to stand overnight at room temp with 2 N CrO_3 in acetic acid. After the excess of oxidizing agent had been decomposed with EtOH (5 ml) for 30 min, the green solution was concentrated under red. press. The residue was extracted with ethyl acetate 3 times. The ethyl acetate extract was washed with 1 N H_2SO_4, $NaHCO_3$ aq and water, and dried over anhydrous Na_2SO_4. The solvent was removed under red. press. to give crystalline solids, which were recrystallized from acetone–n-hexane to afford VIII as colourless needles (0·35 g), m.p. 160–165°. UV λ_{max}^{EtOH} 255 mμ (ε 14600). IR ν_{max}^{KBr} cm^{-1}: 3400, 1770, 1740, 1710, 1615. NMR $\delta_{ppm}^{CDCl_3}$: 1·06 (6H), 1·40 (3H), 2·05 (3H), 2·10 (3H), 4·57 (1H), 4·97 (1H, quartet; J_{AX} 3·8, J_{BX} 5·5 c/s). (Found: C, 67·73; H, 7·83. $C_{22}H_{30}O_6$ requires: C, 67·67; H, 7·74%.)

Ethylidene G-I (X). Anhydrous $ZnCl_2$ (250 mg) was added to the solution of G-I (I; 200 mg) in 80% acetaldehyde (5 ml) and left overnight at room temp. Acetaldehyde was removed under red. press. without heating. The residue was extracted with ethyl acetate, the extracts were washed with $NaHCO_3$ aq and water, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a colourless oily product which recrystallized twice from acetone–n-hexane when X was obtained as colourless needles (100 mg), m.p. 152–156°. IR ν_{max}^{KBr} cm^{-1}: 3476, 3388, 1746, 1736, 1723, 1658, 1239. (Found: C, 65·45; H, 8·60. $C_{24}H_{36}O_7$ requires: C, 65·73; H, 8·73%.)

3-*Keto ethylidene* G-I (XI). A solution of X (200 mg) in pyridine (3 ml) was added to CrO_3 (100 mg)–pyridine (2 ml) complex and allowed to stand overnight at room temp. The reaction mixture was poured into ice water and extracted with ethyl acetate. The extracts were washed 3 times with water, dried over anhydrous Na_2SO_4, and the solvent was removed under red. press. to give a crystalline residue which upon recrystallization from acetone–n-hexane afforded XI as colourless needles (150 mg), m.p. 236° (dec). IR ν_{max}^{KBr} cm^{-1}: 3581, 3534, 3447, 1739, 1729, 1257, 1247. (Found: C, 65·99; H, 8·27. $C_{24}H_{36}O_7$ requires: C, 66·03; H, 8·31%.)

Diethylidene G-III (XII). Anhydrous $ZnCl_2$ (3 g) was added to the solution of G-III (III; 1 g) in 80% acetaldehyde (40 ml) and left overnight at room temp. Acetaldehyde was removed under red. press. without heating. The residue was extracted with ether, the ethereal solution was washed with $NaHCO_3$ aq and saline and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave XII (0·75 g) which could not be crystallized.

3-*Keto diethylidene* G-III (XIII). A pyridine solution (10 ml) of amorphous XII (650 mg) was added to CrO_3 (500 mg)–pyridine (10 ml) complex and left overnight at room temp. The reaction mixture was poured into ice water and extracted with ether. The ethereal extracts were washed with 2 N H_2SO_4, $NaHCO_3$ aq and water, dried over anhydrous Na_2SO_4, and then evaporated to dryness. The resulting crystalline residue after recrystallization from acetone–n-hexane afforded XIII as colourless needles (500 mg), m.p. 240–245° (subliming at 210°). IR ν_{max}^{KBr} cm^{-1}: 3446, 1747. NMR $\delta_{ppm}^{CDCl_3}$: 0·97 (3H), 1·04 (3H), 1·25, 1·33, 1·40 (12H), 4·26 (1H, quartet; J_{AX} 2, J_{BX} 4·4 c/s), 4·51 (1H), 4·94 (1H), quartet; J 5 c/s), 5·16 (1H, quartet; J 5 c/s). ORD (in MeoH, $c = 0·51$): $[\phi]_{318}^{trough}$ −5300°, $[\phi]_{274}^{Peak}$ +5350°. (Found: C, 68·65; H, 8·60. $C_{24}H_{36}O_6$ requires: C, 68·54; H, 8·63%.)

α,β-Unsaturated ketoacid (XV). The lactone (VIII; 400 mg) in a mixture of MeOH (15 ml) and water (5 ml) was refluxed with 1N NaOH (1·8 ml) for 15 min. Water was added to the reaction mixture which was then concentrated to half volume under red. press. Further addition of water followed by concentration *in vacuo* and acidification afforded crystalline solids which, after being allowed to

stand overnight in the freezer were filtered off and dried; recrystallization from MeOH–ethyl acetate gave XV as needles (100 mg), m.p. 193–195°. Its IR spectrum was identical with that of the α,β-unsaturated ketoacid derived from G-I (I).[2]

α,β-Unsaturated ketoacid (XVII). A solution of 200 mg of VIII was refluxed for 45 min in 8 ml EtOH and 2 ml 10 N KOH, treated with 5 ml of water and refluxed for a further 10 min, and the solution was concentrated to a small volume (5 ml) under red. press. Addition of water followed by concentration under red. press. was repeated once more. After cooling to room temp the aqueous solution was acidified with 2 N H_2SO_4 (9 ml) and left overnight in the freezer. The white solid which separated was filtered off, washed with cold water and then dried; recrystallization from ethyl acetate afforded XVII as colourless crystals (80 mg), m.p. 160–164°. UV λ_{max}^{EtOH} 260 mμ (ε 12000). IR ν_{max}^{KBr} cm^{-1}: 3370, 1715, 1700, 1598. (Found: C, 65·64; H, 8·11. $C_{20}H_{30}O_6$ requires: C, 65·55; H, 8·28%.)

α,β-Unsaturated ketolactone (XVIII). The ketoacid (XVII; 70 mg) was dissolved in acetic acid (2 ml) and heated for 45 min on a boiling bath. The solution was evaporated to dryness under red. press., and the resulting amorphous residue was recrystallized twice from ethyl acetate when XVIII was obtained as colourless crystals, m.p. 221–224°. UV_{max}^{EtOH} 255·5 mμ (ε 14600). IR ν_{max}^{EtOH} cm^{-1}: 3420, 1780, 1702, 1605. (Found: C, 69·11; H, 8·14. $C_{20}H_{28}O_6$ requires: C, 68·94; H, 8·10%.)

Rearranged ketonic compounds (XXI and XXII). *p*-Toluene-sulphonyl chloride (0·5 g) was added to an ice-cold solution of G-I (I; 1 g) in pyridine (10 ml). The mixture was left for 2 weeks at room temp, poured into ice-water and extracted with ethyl acetate, and the extract was washed with 1 N H_2SO_4, $NaHCO_3$ aq and water, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent under red. press. afforded an amorphous product which formed crystals (420 mg) from ethyl acetate–n-hexane; recrystallization from the same mixed solvent gave XXII as colourless needles, m.p. 140–144°. IR ν_{max}^{KBr} cm^{-1}; 3644, 3446, 3369, 1714, 1695, 1271. NMR $\delta_{ppm}^{CDCl_3}$: 1·10 (3H), 1·17 (3H), 1·30 (3H), 1·37 (3H), 2·15 (3H), 3·59 (1H, triplet; J 5 c/s), 6·22 (1H). ORD (in dioxan, c = 0·57): $[\phi]_{325}^{trough}$ −265°, $[\phi]_{290}^{peak}$ +473°. (Found: C, 64·20; H, 8·69. $C_{22}H_{34}O_6 \cdot H_2O$ requires: C, 64·05; H, 8·80%.)

The mother liquor was concentrated under red. press. and chromatographed on silicic acid. Benzene–ethyl acetate (4:1) eluted XXIII (70 mg) (described later). Benzene–ethyl acetate (3:2) eluted XXI (15 mg) which after recrystallization from acetone–n-hexane afforded colourless needles, m.p. 180–181°. IR ν_{max}^{KBr} cm^{-1}: 3396, 1728, 1685, 1262. NMR $\delta_{ppm}^{CDCl_3}$: 1·08 (3H), 1·17 (3H), 1·36 (3H), 1·53 (3H), 1·96 (3H), 3·65 (1H, quartet; J_{AX} 8, J_{BX} 5 c/s), 4·42 (1H, doublet; J 5 c/s). ORD (in dioxan, c = 0·56): $[\phi]_{321}^{trough}$ −1630°, $[\phi]_{294}^{peak}$ +737°. (Found: C, 70·52; H, 8·71. $C_{22}H_{32}O_5$ requires: C, 70·18; H, 8·57%.)

Rearranged ketonic compounds XXIII *and* XXIV. *p*-Toluenesulphonyl chloride (1·1 g) was added to an ice-cold solution of G-I (I; 1 g) in pyridine (10 ml). The mixture was kept at 0° for 40 hr, then heated at 100° for 1 hr at first and then for a further 5 min after addition of water (5 ml). After cooling, the reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed successively with 1 N H_2SO_4. Evaporation of the solvent under red. press. afforded an amorphous product which upon crystallization from EtOH–n-hexane gave XXIV (540 mg) as colourless needles, m.p. 146–148°. UV λ_{max}^{EtOH} 227 mμ (ε 11000). IR ν_{max}^{KBr} cm^{-1}: 3560, 3415, 1747, 1724, 1705, 1602, 1194, 1180. NMR $\delta_{ppm}^{CDCl_3}$: 0·88 (3H), 1·03 (3H), 1·13 (3H), 1·32 (3H), 2·10 (3H), 2·42 (3H), 4·42 (1H, triplet; J 7 c/s), 6·18 (1H), 7·33 (2H, doublet; J 8 c/s), 7·77 (2H, doublet; J 8 c/s). ORD (in dioxan, c = 0·30): $[\phi]_{318}^{trough}$ −1480°, $[\phi]_{275}^{peak}$ +3200°. (Found: C, 63·76; H, 7·51. $C_{29}H_{40}O_6S$ requires: C, 63·49; H, 7·35%.)

The mother liquor was concentrated under red. press. and chromatographed on silicic acid. Benzene–ethyl acetate (4:1) eluted XXIII (80 mg) as colourless needles, m.p. 138–139°. UV λ_{max}^{EtOH} 227 mμ (ε 11700). IR ν_{max}^{KBr} cm^{-1}: 1731, 1707, 1600, 1259, 1188, 1175. NMR $\delta_{ppm}^{CDCl_3}$: 1·00 (3H), 1·12 (3H), 1·31 (3H), 1·56 (3H), 1·98 (3H), 2·48 (3H), 4·43 (1H, doublet; J 4·7 c/s), 44·44 (1H, quartet; J_{AX} 5·5, J_{BX} 4·8 c/s), 7·35 (2H, doublet; J 9 c/s), 7·82 (2H, doublet; J 9 c/s). ORD (in dioxan) c = 0·34): $[\phi]_{319}^{trough}$ −3090°, $[\phi]_{278}^{peak}$ +3990°. (Found: C, 65·83; H, 7·40. $C_{29}H_{38}O_7S$ requires: C, 65·64; H, 7·22%.)

Further elution with benzene–ethyl acetate (4:1 to 7:3) gave XXIV, (130 mg).

Anhydrous ketone (XXV). A solution of XXIV (300 mg) in collidine (50 ml) was refluxed for 4 hr in a current of N_2. After cooling it was poured into dilute H_2SO_4, left overnight, and then

extracted with ethyl acetate. The usual working up of the extract gave a solid which was chromatographed on alumina. Benzene–n-hexane (3:1) eluted a crystalline solid, which when recrystallized from EtOH–water afforded XXV (20 mg), m.p. 134–136°. UV λ_{max}^{EtOH} 220 mμ (ε 1400). IR ν_{max}^{KBr} cm^{-1}: 1706, 1658, 1644. (Found: C, 80·22; H, 8·94. $C_{20}H_{26}O_2$ requires: C, 80·49; H, 8·78%.)

ORD of 14-*ketone* (XXVII)[1,2] *in dioxan* ($c = 0·1$). $[\phi]_{406}$ −50°, $[\phi]_{367}$ −145°, $[\phi]_{335}$ −778°, $[\phi]_{320}$ −2150°, $[\phi]_{317·5}$ −2660°, $[\phi]_{315}$ −3020°, $[\phi]_{312·5}$ −3130° (trough), $[\phi]_{310}$ −2690°, $[\phi]_{307·5}$ −1880°, $[\phi]_{305}$ −1390°, $[\phi]_{302·5}$ −1070°, $[\phi]_{300}$ −350°, $[\phi]_{295}$ +1320°, $[\phi]_{290}$ +2250°, $[\phi]_{285}$ +3010°, $[\phi]_{280}$ +3440° (peak), $[\phi]_{275}$ +3340°. (Fig. 1).

Phenylglyoxylate (XXX). An ice-cold solution of isopropylidene G-I[6] (1·5 g) in pyridine (8 ml) and benzene (12 ml) was treated dropwise with a solution of phenylglyoxyl chloride (0·83 g) in benzene (4 ml). White pyridinium chloride precipitated after addition of the acyl chloride. The reaction mixture was allowed to stand overnight at room temp. Water was then added to decompose excess of the acid chloride, and the aqueous solution was extracted with ether. The ethereal extract was washed with dil. acetic acid, water, and then dried over Na_2SO_4. The solvent was evaporated to give an oily product, which was chromatographed on silicic acid. The n-hexane–benzene (1:4) elute afforded XXX as an amorphous product (0·64 g). IR ν_{max}^{KBr} cm^{-1}: 1734, 1699, 1627, 1599, 1583, 752, 712, 684. UV λ_{max}^{EtOH} 253 mμ (ε 10900).

Prelog's asymmetric synthesis with ester XXX. MeI (1 g) in absolute ether (5 ml) was added slowly with stirring to Mg (0·2 g) covered with absolute ether and the mixture refluxed for 1·5 hr. The resulting solution was cooled in an ice bath and treated dropwise with XXX (1 g) dissolved in a mixture of absolute ether (10 ml) and benzene (10 ml). The mixture was stirred for 2 hr at room temp and then refluxed for 1·5 hr with stirring. The complex was decomposed with ice and dilute aqueous acetic acid to give a clear solution which was extracted 3 times with ether. The ethereal extracts were washed with saline and evaporated to give an oily product, which was refluxed for 5 hr with KOH (1·5 g) in MeOH (20 ml) and benzene (5 ml) under N_2 atm. The solvents were evaporated under red. press. and treated with water. The aqueous solution was washed 3 times with ether, acidified with 6 N HCl, saturated with NaCl, and again extracted thrice with ether. Evaporation of the ether afforded faintly coloured crystals of atrolactic acid. Recrystallization from ligroin gave needles (130 mg; 43%), m.p. 97–97·5°. $[\alpha]_D$ ±0° (in EtOH, $c = 6·49$).

3-*Benzoyl* G-I (XXXII). Benzoyl chloride (0·352 g) was added to an ice-cold pyridine solution (5 ml) of G-I (1·2 g) and left for 9·5 hr at room temp. The reaction mixture was treated with ice water and was extracted with ethyl acetate after being allowed to stand overnight at room temp. The organic extract was washed with dil. H_2SO_4, $NaHCO_3$ aq and water, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent under red. press. gave an amorphous product which crystallized from benzene. Additional crystals were obtained from the mother liquor. The combined crystals were recrystallized from acetone–n-hexane to give XXXII as colourless needles (400 mg), m.p. 215–218° (dec). IR ν_{max}^{KBr} cm^{-1}: 3544, 3074, 1736, 1723, 1710, 1603, 1585, 1283, 1231, 713. $[\alpha]_D$ +13·5° ($c = 3·16$, in dioxan). (Found: C, 68·01; H, 7·57. $C_{29}H_{40}O_8$ requires: C, 67·42; H, 7·80%.)

3-*Benzoyl*-6-*acetyl* G-I (XXXIII). The benzoate (XXXII; 20 mg) was allowed to stand overnight at room temp with acetic anhydride (1 ml) and pyridine (1 ml). The reaction mixture was poured into ice water and extracted with ethyl acetate. The extract was washed with dil. H_2SO_4. Evaporation of the solvent under red. press. gave an amorphous product XXXIII. IR ν_{max}^{Nujol} cm^{-1}: 3570, 3450, 1745, 1720, 1604, 1585, 1248, 712.

The IR spectrum of XXXIII was identical with that of the compound obtained from benzoylation of the 6-acetate XXXI.

A. *Horeau's asymmetric method*

(a) α-*Phenylbutyric anhydride*. Racemic α-phenylbutyric acid was refluxed in acetic anhydride for 6 hr. Evaporation of the solvent *in vacuo* gave the oily anhydride.

(b) *General procedure*. The alcohol (0·1 mmole) was dissolved in pyridine (0·3 ml) and left for 15 hr at room temp with α-phenylbutyric anhydride (0·3 mmole). The reaction mixture was then heated with one drop of water for 30 min at 100°; benzene was added and the mixture was titrated with 0·1 N NaOH in the presence of phenolphthalein. The aqueous layer was separated from the benzene layer, washed with benzene, acidified with dil. H_2SO_4, and then reextracted with benzene.

The benzene extract was washed with water and evaporated under red. press. to give α-phenylbutyric acid as an oily product. The optical rotation (α_D) of the benzene solution (1 ml) of the acid was measured in a 0·5 dm cell.

(c) *Results*

	A	B	C	D
XVI	55%	−0·40° (±0·01°)	92%	+35·8
XXXIV	55%	−0·29° (±0·02°)	67%	−41·9
XXXV	40%	+0·06° (±0·01°)	19%	−45·9
I (G-I)	61%	+0·11° (±0·01°)	23%	±0

A: Percentage of esterification.
B: Observed rotation measured in a 0·5 dm cell.
C: Optical yield.
D: Optical rotation $[\alpha]_D$ of the alcohol in dioxane.

3-*Ketodiacetyl* G-III (XXXVI). 3-Keto G-I, m.p. 260°, prepared by N-bromosuccinimide oxidation of G-I,[8] was acetylated with acetic anhydride and pyridine, worked up as usual, and recrystallized from acetone–n-hexane, m.p. 187–190°. (Found: C, 63·88; H, 8·08. $C_{34}H_{36}O_8$ requires: C, 63·70; H, 8·02%.)

ORD: (in MeOH, $c = 0{\cdot}051$): $[\phi]_{315}^{\text{trough}}$ −2370°, $[\phi]_{275}^{\text{peak}}$ +3800°.

3-*Ketoisopropylidenedianhydro* G-I (XXXVII). The compound was prepared according to Ref. 2, m.p. 180°.

ORD: (in dioxan, $c = 0{\cdot}051$): $[\phi]_{321}^{\text{trough}}$ −8200°, $[\phi]_{280}^{\text{peak}}$ +3650°.

Acknowledgment—The authors are greatly indebted to Dr. Mishima, Sankyo Pharmaceutical Co., Tokyo, for generously providing us samples of grayanotoxin-II.