

STRUCTURES OF KAURANOIC ACIDS IN CALONYCTION ACULEATUM

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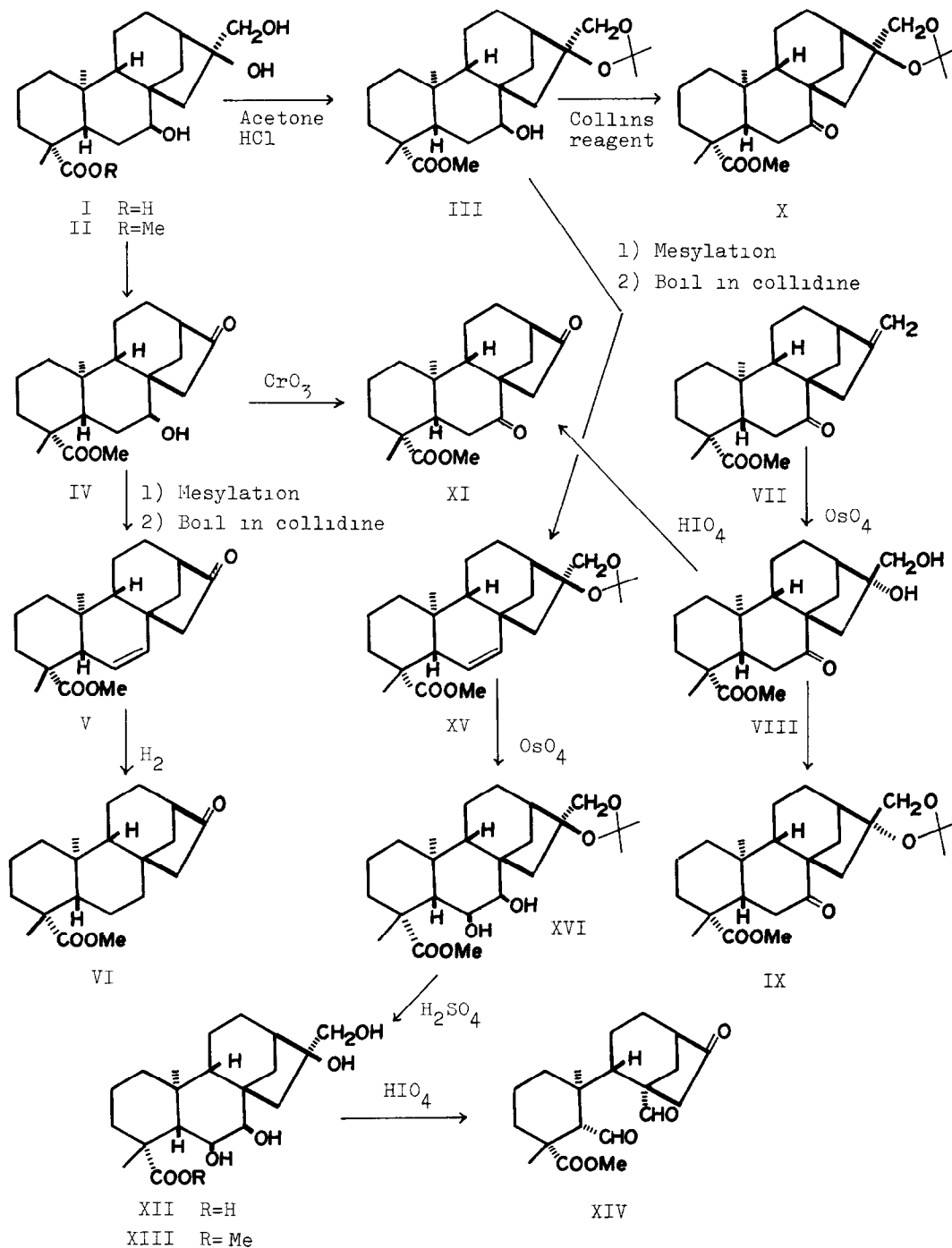
During the isolation of gibberellins from immature seeds of evening-glory (Calonyction aculeatum), we isolated two diterpenoid acids. Here we report structural determination of these substances.

The ethyl acetate-soluble acidic material from immature seeds of evening-glory (23 kg) was purified by countercurrent distribution using ethyl acetate-phosphate buffer (pH 5.4) and silicic acid column chromatography successively to afford two substances, mp 290-292° (74 mg), and mp 260-262° (480 mg), which were tentatively named A and B, respectively.

Treatment of A with diazomethane afforded the monomethyl ester (A-Me, II), mp 224-226°. The molecular formulas  $C_{20}H_{32}O_5$  and  $C_{21}H_{34}O_5$  were given to A and A-Me on the basis of elemental analyses and mass spectra. The NMR spectrum of II in deuteropyridine reveals three 3H singlets at  $\delta$  1.09 (tert  $\underline{CH}_3$ ), 1.32 (tert  $\underline{CH}_3$ ), 3.67 ( $COO\underline{CH}_3$ ) and a 2H singlet at  $\delta$  3.84 ( $-\overset{|}{C}-\underline{CH}_2OH$ ) but no olefinic proton signal is observed. The presence of a system  $>C-\overset{OH}{\underset{CH_2OH}{|}}$  was indicated by formation of the acetonide (III) and rapid consumption of periodate to give the monohydroxy-norketoester (IV). IV reveals a 1H triplet at  $\delta$  3.74 in its NMR spectrum ( $CDCl_3$ ) with a small half width ( $W_{1/2}=7$  Hz), suggesting an axial nature of the secondary hydroxyl group. Treatment of the mesylate of IV with boiling collidine afforded the unsaturated ketoester (V) by the elimination of the axial hydroxyl group in IV. V shows two olefinic proton signals at  $\delta$  5.40 and 6.13 with mutual coupling ( $J=10$  Hz) and the former signal splits further with  $J=4$  Hz and the latter with  $J=2$  Hz (an allyl coupling). The coupling nature in-

indicates the presence of a system  $-\overset{|}{\text{C}}\text{H}=\text{CH}=\overset{|}{\text{C}}-$  in V. V was hydrogenated over  $\text{PtO}_2$  to afford the saturated norketoester (VI), which was identical with methyl ent-16-oxo-17-norkauran-19-oate kindly provided by Dr. K. Mori. These evidences indicate that A must have a structure containing one additional hydroxyl group in ent-16,17-dihydroxykauran-19-oic acid. Since NMR data and melting point of V are in good agreement with those in the literature<sup>1)</sup>, the double bond in V exists in  $\text{C}_6-\text{C}_7$ . The axial nature of the carbonyl proton of IV suggests that the hydroxyl group attaches at  $\text{C}_6\alpha$  or  $\text{C}_7\beta$ . The latter case must be preferred since A could not be lactonized under any condition. The configuration of  $\text{C}_{16}$ -substituents was established from the following reaction sequence Methyl ent-7-oxo-16-kauran-19-oate (VII)<sup>2)</sup> prepared from 7-hydroxykaurenolide was converted to the dihydroxy-compound (VIII) with osmium tetroxide. The acetonide (IX) of VIII shows a similar mass spectrum to that of X, the oxidation product of III with Collins reagent, but their IR and NMR spectra are clearly different, suggesting the epimeric relationship only at  $\text{C}_{16}$  between IX and X<sup>3)</sup>. This was confirmed by conversion of VIII with periodate and IV with  $\text{CrO}_2$  to the identical diketoester (XI). Since it has been known<sup>4)</sup> that osmilation of the  $\text{C}_{16}$ -exomethylene in ent-kaurene derivatives results in the formation of the  $\text{C}_{16}\alpha$ -hydroxyl and  $\text{C}_{16}\beta$ -hydroxymethyl groups, A must have the  $\text{C}_{16}\beta$ -hydroxyl group. Thus the structure I, ent-7 $\alpha$ ,16 $\alpha$ ,17-trihydroxykauran-19-oic acid, is assigned to A.

B has the molecular formula,  $\text{C}_{20}\text{H}_{32}\text{O}_6$ , which was deduced by elemental analyses and mass spectra of B and its monomethyl ester, mp 190-191°, (XIII). The NMR spectrum of XIII in deuteropyridine resembles that of A-Me, only differences being observed in the down-field shift ( $\delta$  1.73) of one of the tert. methyls and the appearance of a new carbonyl proton at  $\delta$  4.64 (1H, double doublet,  $J=11$ , 2.5 Hz). These data suggest that B probably has the structure containing one additional hydroxyl group in A. XIII was treated with periodate to give the nor-derivative,  $\text{C}_{20}\text{H}_{28}\text{O}_5$ , (XIV). Its NMR spectrum reveals two aldehydic protons which appear as a singlet at  $\delta$  9.86 and a doublet at  $\delta$  9.92 ( $J=2$  Hz). This indicates the presence of the aldehydic functions at  $\text{C}_5$  and  $\text{C}_8$  in XIV, and hence, the existence of  $\text{C}_6$ - and  $\text{C}_7$ -hydroxyl groups in B. The complete structure of B



was confirmed by conversion of A to B. The acetonide III was dehydroxylated by treatment of its mesylate with boiling collidine to afford XV, which was osmilated to give the C<sub>6</sub>β,C<sub>7</sub>β-diol (XVI), where the reagent is expected to attack from β-side. The acetyl group was removed with treatment of ethanediol containing a trace of sulfuric acid to give B-Me (XIII). Thus the structure of B was established as XII, ent-6α,7α,16α,17-tetrahydroxykauran-19-oic acid. The down-field shift of one (C<sub>18</sub>) of tert. methyl signals in the NMR spectrum of XIII can be explained by the deshielding effect of C<sub>6</sub>β-hydroxyl group in proximity.

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#### References and Note

- 1) B. E. Cross and J. C. Stewart, Phytochem., 9, 1065 (1970).
- 2) R. H. B. Galt and J. R. Hanson, Tetrahedron, 22, 3185 (1966).
- 3) Methyl ent-7α-hydroxy-16-kauren-19-oate kindly provided by Dr. J. MacMillan was osmilated to give the trihydroxy-ester which shows a similar mass spectrum to that of II but its retention time on GC differed from that of II. This also suggests the epimeric relationship at C<sub>16</sub> between both compounds.
- 4) C. A. Henrick and P. R. Jefferies, Aust. J. Chem., 17, 915 (1964).