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SHORT COMMUNICATION

THE BIOLOGICAL ACTIVITY OF GIBBERELLIN ANALOGUES: ZIZANOIC ACID

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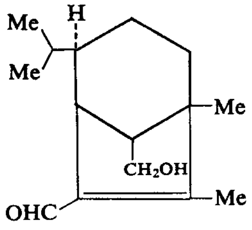
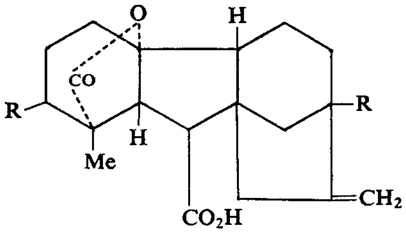
Abstract—Zizanoic acid has been identified as a constituent of vetiver oil from Zambia. It has no plant-growth regulating activity.

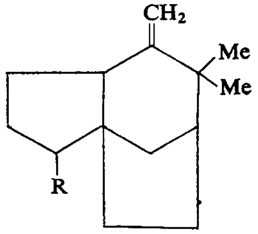
THE SESQUITERPENE alcohol helminthosporol (I) (and certain close analogues and derivatives) shows weak activity in some of the bioassays for gibberellin-like plant-growth regulation; in others it is without activity.[1] Helminthosporol bears a superficial structural relationship to rings CD of the gibberellins (II; R = H or OH) and this has led to the controversial suggestion[2] (cf. Ref. 3) that plant-growth regulation in gibbanes derives from the presence of the bicyclo-[3,2,1]-octane portion of the molecule.



Helminthosporol
(I)



(II)



Zizanoic Acid
(III)
R = CO_2H

Structure III (R = CO_2H), recently assigned[4] to the sesquiterpene zizanoic acid, bears a closer relationship than helminthosporol to rings BCD of the gibberellins: in particular, the five-membered ring fused to the bicyclooctane system carries a carboxyl substituent, a feature essential to high activity in the gibberellins.[3] Zizanoic acid is a constituent of the

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[2] D. E. BRIGGS, *Nature* **210**, 418 (1966).
[3] P. W. BRIAN, J. F. GROVE and T. P. C. MULHOLLAND, *Phytochem.* **6**, 1475 (1967).
[4] F. KIDO, H. UDA and A. YOSHIKOSHI, *Tetrahedron Letters* 2815 (1967).

essential oil of vetiver, *Vetiveria zizanoides*, cultivated in Japan and is considered to be identical with vetivenic acid[5] and khusenic acid[6] isolated by different workers from vetiver oil obtained from other sources; it is related to khusimol[7] (III; R = CH$_2$OH) and tricyclovetivene (zizaene)[8] (III; R = Me), well-known constituents of the neutral fraction of vetiver oil but hitherto allocated incorrect structures.

The composition of vetiver oil varies with the country of origin.[9] We have identified zizanoic acid* (as its methyl ester) as the major component (75 per cent) of the acid fraction (0·42 per cent) of a sample of vetiver oil originating from Zambia. Zizanoic acid had no gibberellin-like activity in the lettuce seedling,[3] cucumber seedling[3] or pea seedling tests[10] for plant-growth regulation. It was also inactive in the *Rumex* leaf senescence[11] and barley endosperm[12] assays.

Structure III (R = CO$_2$H) may not however be intrinsically inactive; the absolute configuration[13] of zizanoic acid may be incompatible with the spatial requirements for activity.

EXPERIMENTAL

Identification of Zizanoic Acid in Vetiver Oil

Oil of vetiver (332 g, ex. Zambia) in ether (350 ml) was extracted with aqu. NaHCO$_3$ (5 × 50 ml). Recovery of the acid fraction furnished a yellow oil (1·27 g, 0·42 per cent) which was methylated in ether with CH$_2$N$_2$ during 18 hr at 20°. The crude esterified product (1·26 g), on recovery, was examined by gas–liquid chromatography (GLC) on a Ucon-oil (50 HB-2000) support coated open tubular column (50′) at 140° in a Perkin-Elmer F11 instrument fitted with a flame-ionization detector. One major component (*ca.* 75 per cent) with a retention time of 108 min was detected together with some twenty-five minor components and trace quantities of other impurities, mainly of shorter retention time.

The crude ester (1·0 g) was distilled *in vacuo*. The i.r. spectrum of the fraction, b.p. 132–140°/3 mm, $[\alpha]_D^{23}$ +43° (453 mg, 0·2 per cent of the vetiver oil), was identical with that of an authentic specimen of methyl zizanoate, ν_{max} (liquid film) 1735 (ester C=O); 3100, 1645, 895 cm^{-1}. ($\rangle$C=CH$_2$). The NMR spectrum (100 Mc) in CDCl$_3$ showed peaks at τ 8·95 (singlet, 6H, $\rangle$CMe$_2$), 7·4 (multiplet, 1H, $\rangle$C*H*·CO$_2$Me), 6·4 (singlet, 3H, OMe) and 5·3, 5·4 (multiplet, 2H, $\rangle$C=CH$_2$), consistent with structure III (R = CO$_2$Me). The purified ester had the same retention time in GLC as the major component of the crude ester and gave a single spot, R_f 0·68 (in u.v. light), in TLC on silica gel HF$_{254}$ (Merck) run in hexane–ether (7:3). Authentic methyl zizanoate had identical GLC and TLC data.

Methyl zizanoate (35 mg) was heated under reflux for 6 hr with N-methanolic KOH (1 ml). Recovery of the acid fraction furnished the oily zizanoic acid (24 mg). The i.r. spectrum (liquid film) was identical with the published data [4,14]

Bioassays

Zizanoic acid was applied at 100, 10 and 1·0 μg/ml (or μg/plant as appropriate) in the lettuce seedling, cucumber seedling, pea seedling, *Rumex* leaf senescence and barley endosperm assays. It had no gibberellin-like activity in any assay. The highest dose was toxic to pea and cucumber and also prevented the response to gibberellic acid in the endosperm assay.

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* Preferred to the earlier synonyms.

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