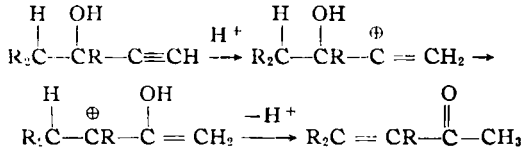
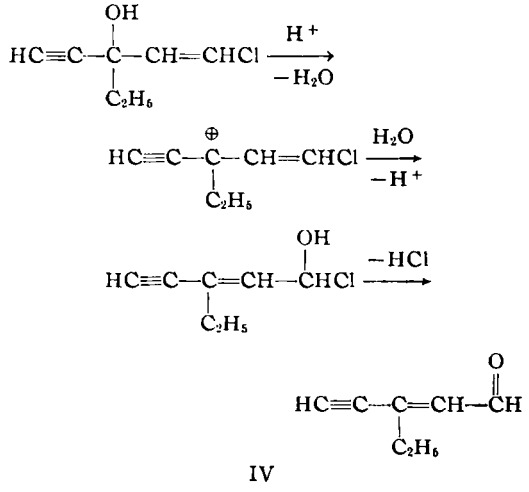


Using an acidic 2,4-dinitrophenylhydrazine reagent (9), rearrangement and derivative formation occurs in one step. Ethinamate and methylparafynol react as expected to yield a derivative of the corresponding α,β-unsaturated methyl ketone. With ethchlorvynol, however, rearrangement to a carbonyl compound seems to proceed through an allylic 1,3-shift of the hydroxyl group with subsequent loss of hydrogen chloride leaving the acetylenic group intact



Analytical and spectral data are in agreement with structure IV. The mode of formation of the compound is analogous to that of 3-methyl-2-penten-4-ynal from 3-methyl-1-chloro-1-penten-4-yn-3-ol by allylic rearrangement (13). The properties of compound IV are compared with those of its homologs in Table II.

Using *p*-nitrobenzoic acid and *p*-toluenesulfonyl chloride in pyridine (8, 15), the tertiary alcohols are readily esterified to form the corresponding derivatives in excellent yield. With ethchlorvynol, yields of about 85% are obtained after heating the reaction solution on a steam bath for 40 minutes or more. Shorter reaction times of 10, 20, and 30 minutes give crude yields of 34, 52, and 72%, respectively.

The above procedures were used successfully for the identification of ethinamate and ethchlorvynol in pharmaceutical preparations. Ethinamate can be readily isolated from tablets by chloroform extraction, removal of the solvent, and crystallization of the residue from petroleum ether. Identification is then made by melting point determination and infrared analysis and confirmed by preparation of the 2,4-dinitrophenylhydrazone derivative. Ethchlorvynol is identified by dissolving the contents of one capsule in a small amount of alcohol and treating with 2,4-dinitrophenylhydrazine reagent. Alternatively, the contents of one capsule may be dissolved in chloroform, the chloroform solution washed with water, dried with anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue readily forms the *p*-nitrobenzoate derivative.

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Communications

Free 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one in Maize

Sir:

Since the discovery that methoxybenzoxazolinone (MBOA) is formed during the extractive treatment of wheat or maize plants (1) from 2,4-dihydroxy - 7 - methoxy - 1,4 - benzoxazin - 3 - one (aglucone), which is enzymatically released from its glucoside when the fresh plant tissue is disintegrated, the presence or absence of MBOA in uninjured maize tissue has been a matter of controversy between Beck *et al.* (2) and ourselves. We found no MBOA when the enzymes of the maize seedlings (Early Albert and a sugar maize variety, grown in a greenhouse) were destroyed by immersing the intact plants in boiling water or in cold alcohol (1). Only small amounts of free aglucone were present in 1 to 2-month-old plants, and free aglucone could not be detected in young seedlings.

The large amounts of MBOA found by Beck *et al.* (2) would imply a qualitative difference in

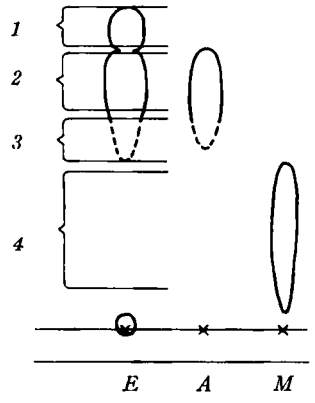

Fig. 1.—Solvent 2% AcOH in water. Key: *E*, plant extract corresponding to 1 Gm. fresh weight; *A*, pure aglucone; *M*, pure MBOA. The contoured spots were dark in U.V. light. Color of aglucone with $FeCl_3$-blue. The numbers on the chromatogram indicate the zones eluted for U.V. spectrometry. Key: *1*, unknown; *2* and *3*, spectrum identical with that of pure aglucone; *4*, no absorption, indicating the absence of MBOA. Concentration of eluate from which U.V. spectrum was measured corresponds to 1 Gm. fresh weight in 3 ml. of ether.

the maize varieties analyzed by us. It was, however, shown that the transformation of free aglucone into MBOA readily takes place in alcohol or in the presence of aluminum oxide (1, 3). The MBOA found by Beck *et al.* after treatment of the intact plants with liquid air and alcohol and chromatography on aluminum oxide could thus have been formed partly or totally from the aglucone, if the plants contained this in free form. In this case, there would be only a quantitative difference between the plant varieties.

To elucidate this problem, the following experiments were made using maize seeds (resistant maize inbred W 22).[1]

I.—The seedlings were grown on the field and harvested when about 7 cm. high.

(*a*) A sample was crushed at about 10°, allowed to warm to room temperature for enzymatic splitting of the glucoside, and extracted with ether. The extract was analyzed for MBOA and the aglucone as described earlier (1) (by paper chromatography). No MBOA was found, but the aglucone was present in an amount of about 700 mcg./Gm. fresh weight.

(*b*) Another sample was immersed in solid CO_2-ethanol mixture immediately after the plants were harvested. The amount of aglucone was about half the amount in experiment *Ia*. Also, traces of MBOA were found.

(*c*) A third sample was immersed in ethanol at room temperature and analyzed as before. This time aglucone and MBOA were found in about equal amounts; the combined amount was of the same order of size as the amount of aglucone in experiment *Ia*.

II.—A second harvest was made when the seedlings were about 20 cm. high.

A sample was crushed and extracted with ether. Aglucone was found, but not MBOA.

III.—To decide whether the MBOA found in experiments *Ib* and *Ic* was present in the plants together with the aglucone or was formed during the analysis, a sample of plants from the second harvest (kept frozen at −20° for about 3 months) was crushed in the frozen state, and the press juice collected at 0 to +3°. The juice was immediately centrifuged at 12000 g. for 30 minutes, between 0 and 2°. The supernatant was freeze-dried; the dry residue, mixed with granular calcium sulfate as a drying agent, was then extracted with dry peroxide and alcohol-free ethyl ether at +10° (270 mm. Hg) for 6 hours. The chromatogram obtained from the concentrated ether extract, with 2% acetic acid in water as solvent, showed the presence of aglucone, but no MBOA. The U.V. spectra obtained on eluting the spots with ether were consistent with this result. On repetition of this experiment, the same result was obtained. The paper chromatogram is shown in Fig. 1.

From these results it is concluded that MBOA is not present in fresh plant tissue even of the resistant maize inbred W 22, but that the free aglucone is present in appreciable concentration in this maize variety and, accordingly, may vary widely in different maize strains. The aglucone which has antifungal properties may thus be the resistance factor in maize (4).

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