GAS CHROMATOGRAPHIC EVIDENCE FOR THE OCCURRENCE OF HOP OIL COMPONENTS IN BEER*

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(Received June 28th, 1965)

INTRODUCTION

Several hop oil components have been found in hopped wort, but most do not survive the brewing process and do not appear in the final product. Identified as being present in the hopped wort were methyl dec-4-enoate and methyl deca-4,8-dienoate[1]. Evidence reported here demonstrates that these compounds probably are trans-esterified and that they appear in the final product as the ethyl esters.

METHOD

We collected steam-volatile, pentane-soluble concentrates from 9 l of commercially prepared wort (before and after hopping), fermented wort, and ale by using a combination distillation–extraction apparatus (see Fig. 1) previously described[1]. The concentrates were evaporated to 200 μl. A sample (4 μl) of the whole concentrate was analyzed by gas chromatography. The oxygenated and hydrocarbon fractions were separated on a silicic acid column (0.5 × 10 cm, 13.5 % moisture). The hydrocarbon fraction was eluted with 10 ml pentane, and the oxygenated fraction eluted with 10 ml diethyl ether. The fractions were concentrated to 200 μl and an aliquot analyzed by gas chromatography. The remainder of the oxygenated fraction was concentrated to about 20 μl and saponified with 50 μl 2 % KOH in 90 % MeOH. The saponification was carried out in a sealed ampoule kept at 100° for 1 h.

The non-saponifiables, including alcohols, were extracted with pentane, concentrated and analyzed by gas chromatography. The aqueous phase was then acidified with a few drops of 10 N H_2SO_4 and extracted with ether. The ether extract was concentrated and pentane added. The solution was divided into 2 parts and evaporated in separate ampoules. Esterification was carried out with approximately 50 μl of methyl or ethyl alcohol containing 2 % H_2SO_4. The ampoule was sealed and kept at 100° for 1 h. A few drops of water were added and the esters were extracted from the

* Technical paper No. 1994 of the Oregon Agricultural Experiment Station, Corvallis, Oregon, Agricultural Chemistry Department, O.A.E.S. and Crops Research Division, Agriculture Research Service, U.S. Department of Agriculture. This work was supported in part by the U.S. Brewers Association.

aqueous phase with pentane. The pentane extract was concentrated to 25 μl and an aliquot taken for gas chromatographic analysis. The procedure followed in separating the concentrates is outlined in Table I.

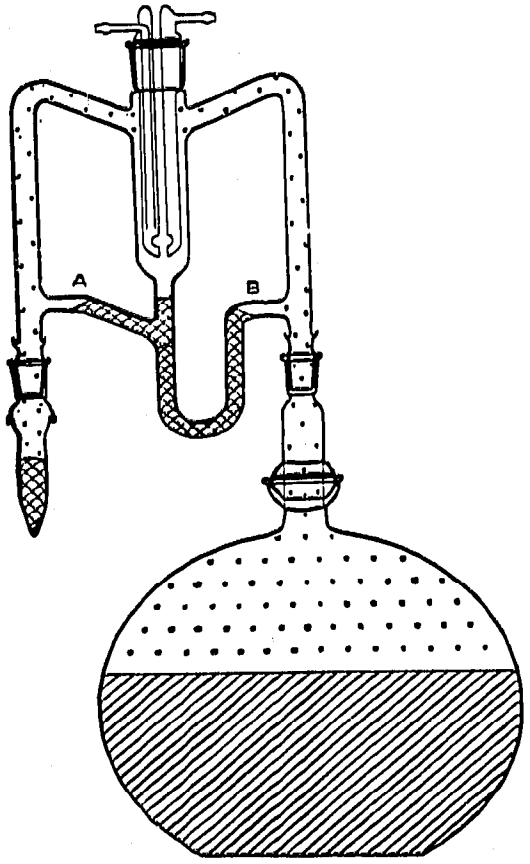

Fig 1. Distillation–extraction apparatus. The steam-volatiles from the sample are extracted continuously in fresh pentane. The aqueous phase is returned to the large distilling flask through arm B. The pentane is returned through arm A and concentrated in the small tube.

Standard ethyl esters were prepared from reference samples of methyl dec-4-enoate and methyl deca-4,8-dienoate supplied by Dr. BUTTERY*.

A 'Late Cluster' hop oil (the variety used in the commercial samples) was separated on a silicic acid column. The methyl and ethyl esters were prepared from the saponified portion of the oxygenated fraction.

Gas-chromatographic separations were made on an $^1/_8$ in. × 27 ft. column packed with 2% BDS coating on 60/80 mesh Chromosorb "P" at 144°, 27.5 p.s.i. N_2 inlet pressure, 1:3 split with hydrogen flame detector.

RESULTS AND DISCUSSION

The commercial brew had been hopped at the rate of 0.5 lb/bbl. Approximately 260 p.p.b. methyl dec-4-enoate and 165 p.p.b. methyl deca-4,8-dienoate were available

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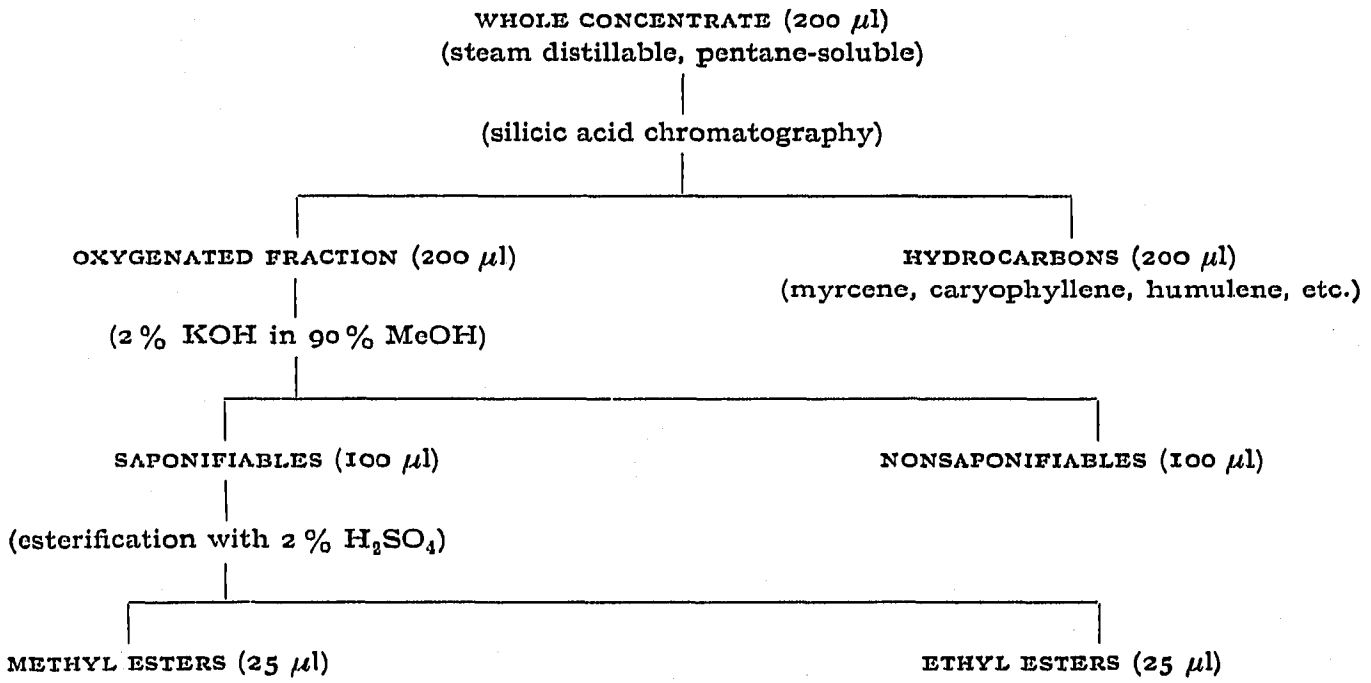
TABLE I

SEPARATION OF CONCENTRATES FROM WORT, FERMENTED WORT, AND ALE



to the wort. According to previous experiments[2,3], only 20 % of the available hop oil remains in the wort. The remainder is lost during boiling or is discarded with the spent hops. Table II shows the amounts found in wort, fermented wort and ale.

Previous results showed that there were no peaks corresponding to methyl dec-4-enoate or methyl deca-4,8-dienoate in unhopped wort[1]. Fig. 2 shows the appearance of these peaks in the oxygenated fraction of hopped wort. Peak number one is methyl dec-4-enoate and number two is methyl deca-4,8-dienoate. (The small peak following number one is undecanone-2, which disappears upon saponification.) These correspond to the methyl esters prepared from Late Cluster hop oil. These peaks have no counterparts in the oxygenated fraction of the ale. However, the two peaks numbered 3 and 4 (ethyl dec-4-enoate and ethyl deca-4,8-dienoate) do correspond to

TABLE II

RECOVERIES OF METHYL AND ETHYL ESTERS OF DEC-4-ENOIC AND DECA-4,8-DIENOIC ACIDS

	Available p.p.b. at 0.5 lb/bbl.	*Approximate p.p.b. recovered*		
		Wort	*Fermented wort*	*Ale*
Dec-4-enoate	260	33[a]	919[b,c]	316[b,c]
Deca-4,8-dienoate	165	31[a]	35[b]	38[b]

[a] As methyl esters.
[b] As ethyl esters.
[c] Interference from fermentation product.

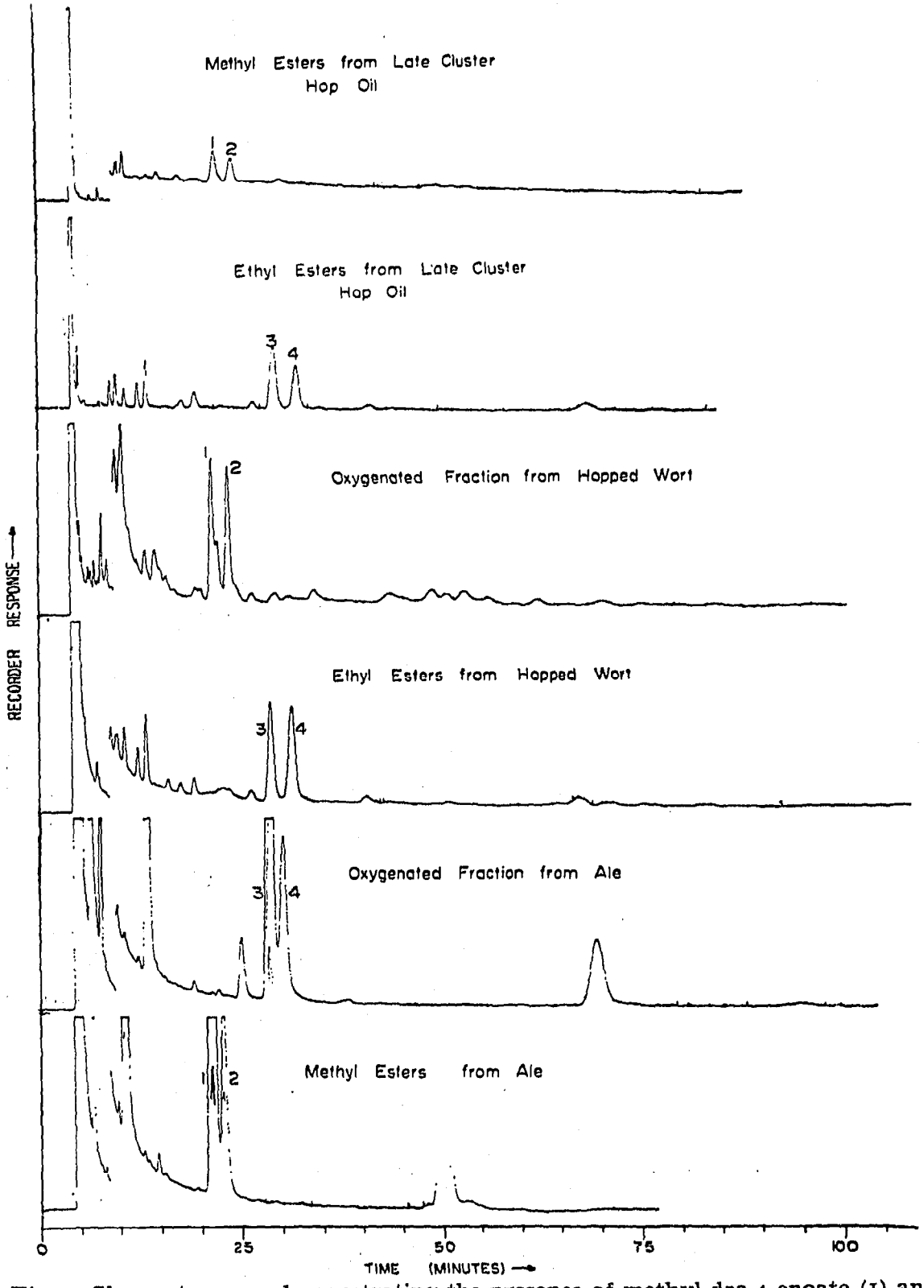


Fig. 2. Chromatograms demonstrating the presence of methyl dec-4-enoate (1) and methyl deca-4,8-dienoate (2) in hop oil and wort, and ethyl dec-4-enoate (3) and ethyl deca-4,8-dienoate (4) in ale.

the ethyl esters prepared from hop oil and hopped wort. For further evidence toward the identity, the methyl esters prepared from the oxygenated fraction of ale are compared to the methyl esters of hop oil and the oxygenated fraction of hopped wort.

Only those compounds that are oxygenated and saponified are possibilities for the peaks found in fermented wort and ale. Comparison of retention times of the ethyl and methyl esters prepared from reference samples, hop oil, wort, and ale show that they are practically identical.

Ethyl deca-4,8-dienoate appears in ale in the same proportion that methyl deca-4,8-dienoate appears in wort (Fig. 2). However, the ethyl dec-4-enoate peak, number three, appears in much higher concentration in ale than the methyl ester in wort. The ester is probably composed of the trans-esterified ester from hop oil plus additional ethyl dec-4-enoate formed during fermentation. The added increment could include ethyl dec-9-enoate, identified in beer by STRATING AND VENEMA[4], which would not be resolved from ethyl dec-4-enoate by this procedure.

CONCLUSION

It has been shown that methyl deca-4,8-dienoate is trans-esterified during fermentation, and that it is present in the final product as ethyl deca-4,8-dienoate. Methyl dec-4-enoate has been identified in the hopped wort and probably appears as the ethyl ester in the final product.

From the data on an ale prepared with a 0.5 lb/bbl. hopping rate, it can reasonably be assumed that a similar transformation occurs in beer hopped at the rate of 0.2 to 0.3 lb/bbl., and that these esters would appear in concentrations of 12 to 20 p.p.b. It has been reported that the flavor threshold of the methyl esters is 10 and 18 p.p.b. in distilled water[5]. It is very likely that these compounds contribute to the hop flavor and aroma in beer.

SUMMARY

Steam-volatile, pentane-soluble concentrates collected from wort and beer were analyzed by gas chromatography. Two hop oil components, methyl dec-4-enoate and methyl deca-4,8-dienoate, were identified in wort. Evidence is presented to demonstrate that these compounds are probably trans-esterified during fermentation, and that they appear in the final product as the ethyl esters.

REFERENCES

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