THE MEASUREMENT OF BITTERNESS IN BEERS

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Up to the present time it has been possible to obtain two different figures for the bitterness of a beer according to whether the analysis has been made and calculated by the Rigby & Bethune Second Method or by the Moltke & Meilgaard and related methods. By co-ordinating the results obtained by the *Iso*humulone Sub-committee of the A.S.B.C. with those of the Analysis Committee of the E.B.C., it has been shown that the results by the two types of method are related in such a way that at 28 parts per million they agree; but, progressively, above this value the Moltke & Meilgaard figures become larger than the Rigby & Bethune ones for the same beer. The opposite is true below 28 p.p.m. Since, nevertheless, a good relationship exists between the results by the two types of method, they could all be stated on a uniform scale. There are arguments in favour of adopting the Rigby & Bethune calculation, and the Societies have agreed to adopt it and to name the results "International Bitterness Units" (in America "*Iso*humulone Bitterness Units"). As a result, inter-continentally agreed Tables are published here for converting Moltke & Meilgaard results (or for converting the ultraviolet measurements directly) into I.B.U.s. For the great majority of beers—those made with fresh or well-stored hops—the results by the two types of method agree, if they have been calculated in this way. However, the E.B.C. Analysis Committee has studied also beers made from old or deteriorated hops and here, even when calculated in the new units, the two types of method give discrepant results. It has been shown by chromatography that the Rigby & Bethune washing of the *iso*-octane extract with acid methanol removes from such old-hop beers another group of compounds—so leaving purified *iso*humulones. However, the compounds removed are also bitter, so that the value of the Moltke & Meilgaard method for such beers is explained. It seems probably that the extra bittering compounds, additional to *Iso*humulones in beers made from deteriorated hops, are water-soluble derivatives of the resin acids.

INTRODUCTION

THE refreshing bitter taste of beers is one of the most important characteristics of this drink, and since the sense of taste, though very delicate, is not a precise or consistently reliable guide, there have been many attempts to find physical or chemical measures of the bitterness of beer.

In 1955 (*Brau. Wiss. Beil.*, 8, 3), P. Kolbach & H. Schilfarth described an extraction of the bitter substances of beer into chloroform followed by weighing the dried extract. This solvent, it was known, extracted other bodies, such as fatty substances, so that it was necessary to introduce a correction from an analysis of the unhopped wort. The quest was taken a stage further by F. L. Rigby & J. L. Bethune (this *Journal*, 1955, 325) when they showed that *iso*-octane extracts of acidified beer diluted with methyl alcohol could be made alkaline and measured in the ultraviolet at 255 mμ. to give a measure (Method I) which agreed with the bitterness of a series of beers as measured by a taste panel. In their Method II, aiming for improved accuracy, Rigby & Bethune washed the *iso*-octane extract with acidified methanol to remove impurities, a treatment which is considered later in this paper. For both methods they calculated the results to *iso*-humulones, as measured by counter-current distribution, and the relationship was carefully established by the use of a long series of beers covering a very wide range of bitterness.

Following the work of Rigby & Bethune, many laboratories adopted their methods and the measurement of the "*iso*humulone content" of beers became a regular routine. However, these methods were lengthy, so A. B. Moltke & M. Meilgaard (*Brygmesteren*, 1955, 12, 23) produced a variation in which the ultraviolet measurement was carried out

at 275 mμ. on the original *iso*-octane extract from the acidified beer without first making it alkaline or washing it. Moltke & Meilgaard also established a calculation from this ultraviolet reading to *iso*humulones by results which will be discussed later. Shortly after this, W. J. Klopper (*Brauwissenschaft*, 1955, 101) introduced a modification of this acid *iso*-octane method, and another was introduced by M. W. Brenner, C. Vigilante & J. L. Owades (*Amer. Brewer*, 1956, 40). In both these modifications, the Moltke-Meilgaard calculation was retained (apart from alterations due to degree of dilution); in the present paper, for convenience, all references to the Moltke-Meilgaard method are intended to refer to these other two methods as well.

Part I

The Establishment of International Bitterness Units

The relationship between the Rigby & Bethune II and the Moltke-Meilgaard measurements.—From what has been said it will be clear that the Rigby & Bethune II method was originally believed to be the most accurate method available for measuring *iso*humulones in beer (apart from counter-current distribution), but it is a lengthy method, so that the quicker alternatives are preferred by many and it is important to know how results by the two types of method are related. It was therefore a very fortunate circumstance that both the *Iso*humulones Sub-committee of the American Society of Brewing Chemists and the Analysis Committee of the E.B.C. decided to make comparative studies of the Rigby & Bethune Method II and of the Moltke & Meilgaard method on a series of beers. The American studies were chiefly on beers of low *iso*-humulone content and the European ones chiefly on beers of high *iso*humulone content. It was therefore a further fortunate circumstance that the two sets of results can, as the result of an agreement between the two Societies, be considered and published together.

Each of the results obtained by one or other of the Committees (and given later in Table II) can be regarded as accurate and reliable since it is the average of determinations made in ten or more laboratories. From Fig. 1 it can be seen that the ten American and the three European average results obtained in this way all clearly fit together to form one striking smooth curve. There therefore seems no doubt that a clear relationship can be established between results by the Rigby & Bethune Method II and those calculated by the Moltke-Meilgaard equation (within the limitations to be discussed later).

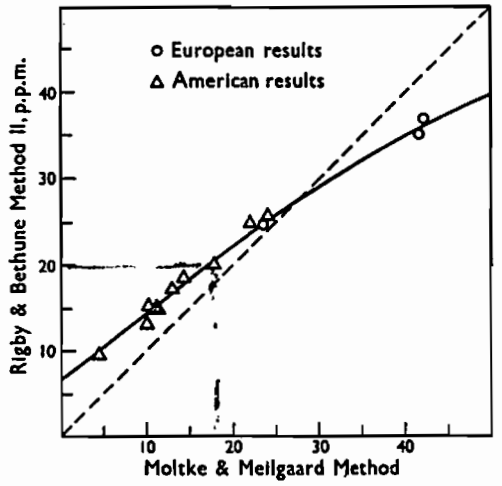

Fig. 1.—*Iso*humulone methods compared.

The dotted line on Fig. 1 shows what would have been obtained if the two methods had given identical results. In fact this dotted line crosses the curve at 28, which shows that at a figure of around 28 the two methods give identical answers. Above that figure the Moltke-Meilgaard method and calculation give progressively larger results than the Rigby & Bethune II and the opposite is true below this figure.

Dr. Rigby was able to explain this discrepancy by pointing out that in his method the results are calculated from an equation derived from studies on beers covering a very wide range of bitterness, whereas the Moltke-Meilgaard counter-current studies were derived from a relatively small number of beers grouped in the middle of the range and the slope of the line was decided by analyses of worts at the two extremes. The Moltke & Meilgaard conversion equation is therefore not considered as accurate as the Rigby & Bethune one. Indeed it seems very probably that this could be substantiated by preparing very lightly hopped beers (say 3–5 I.B.U.s) since these would almost certainly give

negative values on the Moltke & Meilgaard calculation.

Joint Action by the A.S.B.C. and the Analysis Committee

As a result of the discussions which followed the findings detailed in the previous paragraph, the *Iso*humulone Sub-committee of the A.S.B.C. and the Analysis Committee have agreed and accepted these findings. They have also agreed that it is highly undesirable to have two distinct sets of units for bitterness in beers when these units agree only at 28 and diverge increasingly on each side of this figure.

Therefore both Societies have decided to adopt the Rigby & Bethune units as the basis for all measurements of bitterness in beers and to designate these "International Bitterness Units." In America they will be known as "*Iso*humulone Bitterness Units," but the values will be identical.

Adopting this system, when the Rigby & Bethune II method is used then the normal calculation for this method is used. However, it is recognized that in many laboratories the quicker Moltke & Meilgaard method will be preferred. It is possible in this case to use the original equation to calculate the Moltke & Meilgaard result and then to convert this to I.B.U.s, and a Table for doing this (Table I) is given.

It will be noted both in Table I and Table III that values are given over only a limited range. Outside these limits the exact relationship has not been determined and values are therefore not given.

The use of Table I can be illustrated by employing it to convert the European and American results from which it was derived. This illustration is given in Table II. The E.B.C. would at the same time like to acknowledge its gratitude to the A.S.B.C. for permission to use their results.

Calculation of Moltke-Meilgaard values followed by conversion with the help of Table I is complicated and it is clear that the simpler way, after using the Moltke & Meilgaard method, is to calculate the I.B.U. value directly from the absorption figure in acid *iso*-octane at 275 mμ. A Table for this conversion has been agreed by both Societies (Table III).

It should be noted here that, as a result of discussions between the Societies, this Table has been slightly modified from the corresponding one printed in earlier copies of the Second Edition of *Analytica*. Anyone who finds a discrepancy between the two can obtain a correcting page for *Analytica* on applying to The Secretary, P.O. Box 455, Rotterdam, Holland.

Conditions for Accuracy

Since methods and units have been agreed internationally to give results on a uniform scale it is important to draw attention to the analytical conditions needed for good agreement in the results obtained.

With both types of method (Rigby & Bethune II and Moltke & Meilgaard) there is a danger of low results from incomplete extraction: it is therefore desirable to test at

TABLE I

CONVERSION OF MOLTKE-MEILGAARD VALUES TO INTERNATIONAL BITTERNESS UNITS*

Moltke-Meilgaard values	0	1	2	3	4	5	6	7	8	9
0	—	7·2	8·0	8·8	9·6	10·3	11·1	11·9	12·6	13·3
10	14·1	14·9	15·8	16·6	17·5	18·3	19·2	20·0	20·8	21·6
20	22·4	23·1	23·8	24·5	25·2	25·9	26·6	27·2	27·9	28·5
30	29·1	29·8	30·4	31·0	31·5	32·1	32·7	33·2	33·8	34·4
40	34·9	35·5	36·0	36·6	37·0	37·5	38·0	38·5	39·0	39·5
	Corresponding International Bitterness Units									

* The Table gives I.B.U.s for whole-number Moltke-Meilgaard values and intermediate ones can be obtained by interpolation.

TABLE II

MOLTKE-MEILGAARD VALUES CONVERTED TO INTERNATIONAL BITTERNESS UNITS AND COMPARED WITH DIRECT RIGBY & BETHUNE II VALUES

Beer	Average Moltke & Meilgaard values	Calculated International Bitterness Units (from Table I)	Directly determined Rigby & Bethune II values
ASBC 1960–61			
Sample AD	18·0	20·8	20·1
BE	11·1	15·0	15·3
CF	10·1	14·2	13·4
ASBC 1961–62			
A	14·4	17·8	18·8
B	11·1	15·0	15·5
C	24·0	25·2	25·9
D	13·0	16·6	17·5
ASBC 1962–63			
A_1	22·0	23·8	25·0
C_1	4·5	10·0	9·8
D_1	10·4	14·4	15·2
E.B.C.			
AC/61/17 Sample X ..	23·5	24·9	24·7
AC/61/31 Sample Y_1 ..	42·1	36·1	36·8
AC/62/7 Sample Y_2 ..	41·5	35·8	35·0

least once a month whether the shaking is sufficient for good extraction by comparing the results obtained with normal shaking time with those by double shaking time. On the other hand, if the shaking is made more vigorous, there is the danger of forming stable emulsions which give high results. This can be overcome by centrifuging, and, where necessary, by the addition of one drop of *Byprox* (sodium secondary alkyl sulphate) to the beer.

With these methods there is also the danger of photo-catalysed oxidation in the solvents used, so that the extractions should be carried out in dim light and each step in the analysis should be carried out immediately after the preceeding one and as rapidly as possible (see H. Lundin, *Proc. Eur. Brew. Conv., Scheveningen*, 1947, 49).

As pointed out by Moltke & Meilgaard, all extractions should be carried out in a room at a constant temperature of 20·0 ± 0·2° C. since the proportion extracted into *iso*-octane is strongly influenced by temperature.

General Remarks

In the past, as new types of measurement have been adopted in brewing, they have been expressed in different units in different parts of the world. It is therefore a matter of considerable satisfaction that agreement has been reached in this instance between the American Society of Brewing Chemists and the European Brewery Convention to define the relationship between the two types of method for measuring bitterness in beer and so to define units of bitterness which are identical on both sides of the Atlantic.

It is hoped that this will help in avoiding confusion when bitterness measurements by different workers are compared, and it is hoped also that it will provide an example which may be followed when future analytical units are defined.

PART II

THE PROBLEM OF BITTERNESS FROM OLD* HOPS

In Part I are detailed the steps by which agreement has been reached between the A.S.B.C. and E.B.C. on the establishment of

* As used in this paper "old" hops means those which have undergone oxidation of the resins, even if this has occurred in a relatively short time. Similarly, hops may remain "fresh" for a long time if they have not undergone ageing or deterioration in the sense of oxidation of the resins.

TABLE III

TABLE OF INTERNATIONAL BITTERNESS UNITS CORRESPONDING TO MOLTKE & MEILGAARD EXTINCTION READINGS

Moltke & Meilgaard* extinction readings at 275 mμ.	0	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
0·2	6·4	6·6	6·8	7·0	7·2	7·4	7·7	7·9	8·1	8·3
0·3	8·5	8·7	9·0	9·2	9·4	9·6	9·8	10·0	10·3	10·5
0·4	10·7	10·9	11·1	11·4	11·6	11·8	12·0	12·2	12·4	12·7
0·5	12·9	13·1	13·3	13·5	13·7	14·0	14·2	14·4	14·6	14·9
0·6	15·1	15·4	15·6	15·9	16·1	16·3	16·6	16·8	17·1	17·4
0·7	17·6	17·8	18·1	18·3	18·6	18·8	19·0	19·3	19·5	19·8
0·8	20·0	2·02	20·5	20·7	20·9	21·1	21·4	21·6	21·8	22·0
0·9	22·2	22·5	22·7	22·9	23·1	23·3	23·5	23·7	23·9	24·1
1·0	24·3	24·5	24·7	24·9	25·1	25·3	25·5	25·7	25·9	26·1
1·1	26·3	26·5	26·7	26·9	27·1	27·2	27·4	27·6	27·8	28·0
1·2	28·1	28·3	28·5	28·7	28·9	29·0	29·2	29·4	29·6	29·8
1·3	29·9	30·1	30·3	30·5	30·7	30·8	31·0	31·1	31·3	31·5
1·4	31·6	31·8	32·0	32·1	32·3	32·5	32·6	32·8	33·0	33·1
1·5	33·2	33·4	33·6	33·7	33·9	34·1	34·2	34·4	34·6	34·7
1·6	34·8	35·0	35·2	35·3	35·5	35·7	35·8	36·0	36·2	36·3
1·7	36·4	36·6	36·7	36·9	37·0	37·2	37·3	37·4	37·5	37·7
1·8	37·9	38·0	38·1	38·3	38·4	38·6	38·7	38·9	39·0	39·1
1·9	39·3	39·4	39·5	39·7	39·9	40·0	40·2	40·3	40·5	40·6
2·0	40·8	41·0	41·1	41·3	41·4	41·6	41·8	41·9	42·1	42·3
	International Bitterness Units									

* Readings by Klopper or Brenner method should be multiplied by two before entering the Table.

international bitterness units and Part I therefore represents the views of both Societies.

Part I deals with the bitterness from fresh hops and in America the concern is almost entirely with such hops; in Europe, older, stored hops may sometimes have to be used and they introduce further problems. These have been studied by the E.B.C. and the results are given here as Part II. The result is that in Part II the conclusions reached are given as the views of the E.B.C. only.

As a result of the work described in Part I it has become possible to analyse a beer both by the Rigby & Bethune II and the Moltke-Meilgaard methods, to give the results in the same units and to find that both results agree within experimental error (as is illustrated in Table II). This however, applies only to beers made using fresh hops. The Analysis Committee has extended its investigations to beers made from hops which had deteriorated either through age or from imperfect storage, and with beers from such hops it has been

found that there is a discrepancy between the results obtained by the two methods, in spite of the fact that they have been calculated in the same international units.

The first observations were made with two commercial beers. Here there were reasons to suppose that the hops had somewhat deteriorated before use and, in agreement with this, the Moltke-Meilgaard results expressed as I.B.U.s were a few units higher than the Rigby & Bethune II results, as is shown in Table IV.

In some countries this may be regarded as an academic problem, but the results in Table IV suggest that it is not entirely so. With such beers there is no longer the possibility of obtaining satisfactory results by both methods of analysis and it is necessary to decide which gives the better results. To make this choice it is necessary to decide which of the methods more accurately assesses the bitterness of beer made with old hops.

The problem facing investigators in this field is that, in attempting to assess bitterness, it is necessary to undertake the very difficult task of arranging a whole series of beers in order of bitterness as assessed by actual tasting experiments. As a basis for their calculations, Rigby & Bethune did this for an extensive series of beers, and their work forms the basis for the general assumption today that, in measuring *iso*humulones, one is measuring the bitterness of a beer. However, all the beers they used were prepared using fresh hops and it is not until the last few years that taste-test studies on beers from old hops have been published.

S. E. Birtwhistle, J. R. Hudson & A. L. Whitear (this *Journal*, 1963, 239) made comparative brewings of fresh and older hops and in four pairs of comparative tastings found that the relative bitterness of a series

TABLE IV

BITTER VALUES OF BEERS FROM SLIGHTLY DETERIORATED HOPS

Beer	Moltke & Meilgaard value	International Bitterness Units*	*Iso*humulones (Rigby & Bethune II)	Difference
AC/63/A	14·1	17·6	15·0	2·6
AC/63/B	25·8	26·4	22·3	4·1

* Calculated from Moltke-Meilgaard value.

TABLE V

BITTER VALUES OF BEERS FROM BADLY DETERIORATED HOPS

Beers	Moltke & Meilgaard value	International Bitterness Units*	*Iso*humulones (Rigby & Bethune II)	Difference
AC/63/13	26·9	27·1	18·6	8·5
AC/64/9	30·3	29·3	23·7	5·6

* Calculated from Moltke-Meilgaard value.

The differences found in Table IV are not large, but, since the figures are the average of results obtained in fourteen laboratories the discrepancies are accepted as real. The t test gives P% as $\sim 0{\cdot}1$ for both beers, so that the difference is very highly significant.

However, to demonstrate the matter beyond any doubt, badly deteriorated hops were used by the Brewing Industry Research Foundation to prepare beers which were analysed by the E.B.C. Analysis Committee. One of these beers was made using 6-year-old hops (AC/63/13) and the other was made using hops which had been stored at a high temperature for 6 months (AC/64/9). It will be seen from the results in Table V that the differences given by the two methods are very much larger and leave no doubt of the discrepancy.

of beers from old and from fresh hops was measured better by the Brenner (Moltke-Meilgaard) method figures than by the Rigby & Bethune Method II figures.

It therefore seems that the quicker and simpler Moltke & Meilgaard method, at least on an empirical basis, gives the better assessment of the bitterness of beers from old hops. If this is so then it is highly desirable to find the reason.

The nature of bitter substances in old hops.

Faced with this discrepancy between the two methods it was important to try to find if there is a sound chemical basis for the results of the empirical tasting tests.

The original work of Rigby & Bethune demonstrated the overwhelming importance of *iso*humulones as the bittering compounds in beers from fresh hops. Despite this, however, there have been signs from the beginning of modern work on hops that the *iso*humulones, derived in boiling solely from the α acids of hops, were not the only bittering compounds. In 1924 Ford & Tait (this *Journal*, 1924, 426) showed that the β fraction of the hop resin contributed a small additional amount of preservative power to that given by the α acids. A year later Wöllmer (*Woch. Brau.*, 1925, **42**, 1) allowed in his formula for a small contribution to bitterness from the β fraction of fresh hops. Subsequent work has indicated oxidation products of the α or β acids as likely sources of bitterness, especially in old hops, *e.g.*, the delta resin of Walker, Zakomorny & Blakebrough (this *Journal*, 1952, 439) or oxidation products of lupulin as suggested by R. O. V. Lloyd (*Proc. Eur. Brew. Conv., Vienna*, 1961, 112). In the same volume L. O. Spetsig (p. 134) gave evidence that, as the humulones and lupulones declined in ageing hops, the content of hulupones (probably formed from lupulones) increased.

These were, however, the results of general studies on hops and cannot be used directly to explain the present results on the analysis of hops by the *iso*-octane group of methods.

Recent findings.

Perhaps the clearest demonstration of the presence in the *iso*-octane extract of a group of bittering substances other than *iso*humulones is given by the work which Dr. W. Goedkoop has undertaken for the Analysis Committee. He has separated the solids of *iso*-octane extracts of beers by the Spetsig method (*Acta. chem., Scand.*, 1962, **16**, 1586), employing reversed-phase chromatography on hydrophobic kieselguhr, with a continuously increasing pH gradient from pH 2·5–7·2.

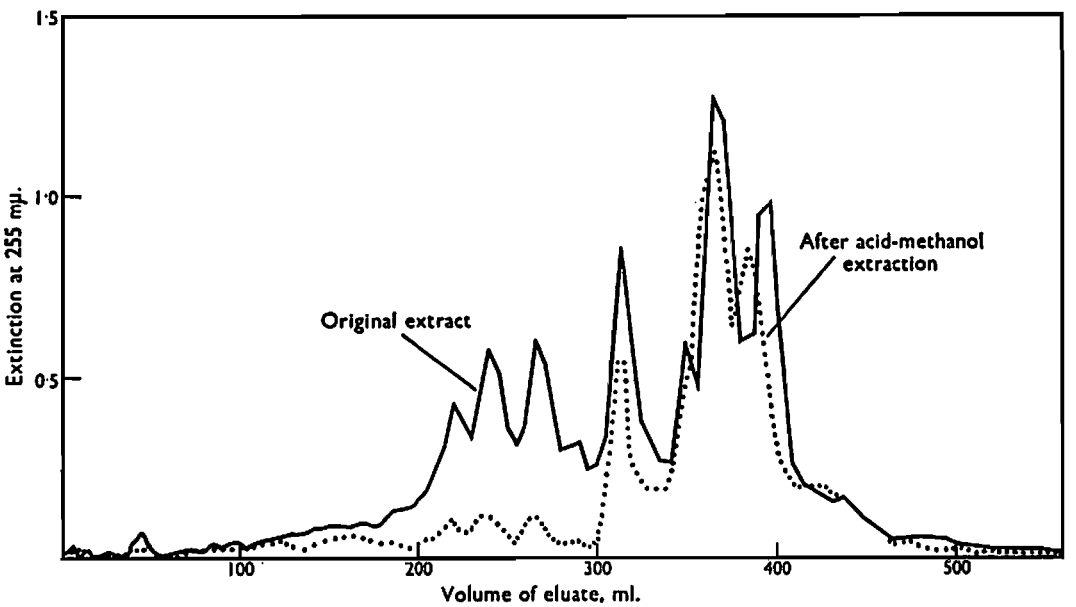

Fig. 2.—Acid-methanol extraction of *iso*-octane extracts of old-hop beer.

The substances eluted were measured by the extinction values at 255 mμ.

As a result, he has been able to study the effect of acid methanol washing on *iso*-octane extracts of beer AC/64/9 in which badly deteriorated hops had been used and his results are illustrated in Figs. 2 and 3. From these it will be seen that the three *iso*-humulones are separated from one another and are eluted at around 340–410 ml. of effluent.

in Fig. 3. Here the chromatographic elution of the extract from the beer prepared from old hops (as in Fig. 2) is compared with the corresponding fractionation of the acid *iso*-octane extract of a Dutch beer made with fresh hops (both without washing of the extract by acid methanol). Both beers are seen to yield the three *iso*humulone peaks. The experimental beer shows (as in Fig. 2) four additional peaks, but in the beer from fresh hops there are only slight indications

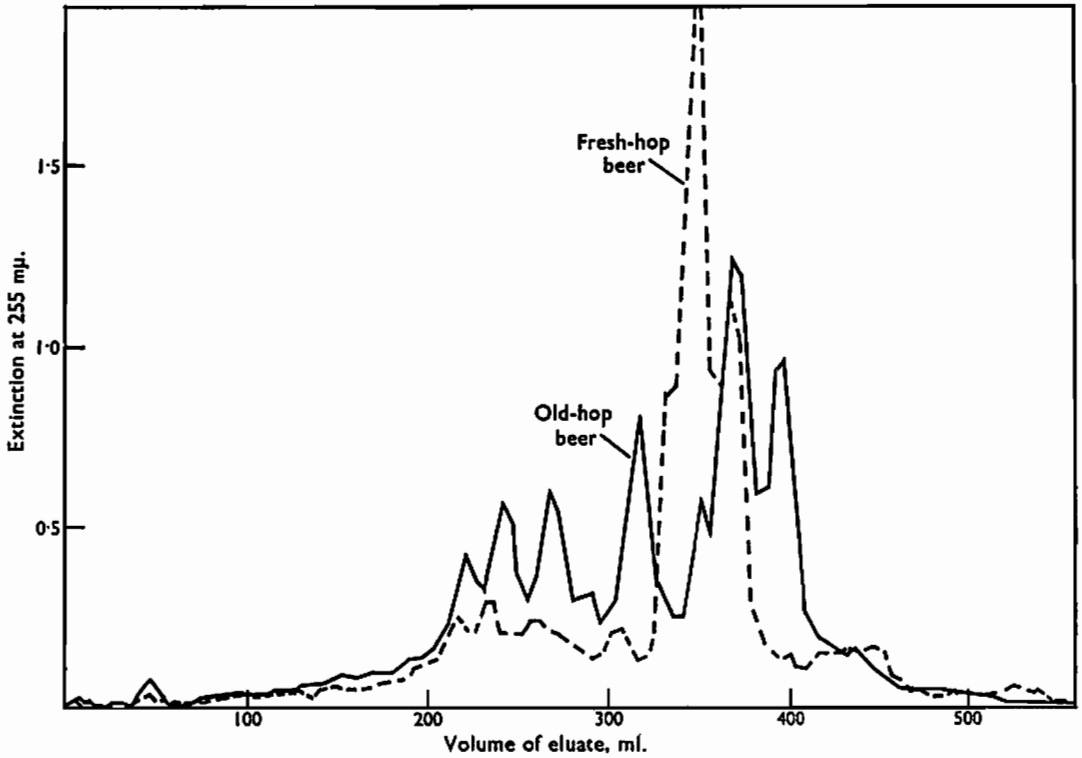

Fig. 3.—*Iso*-octane extracts of old-hop and fresh-hop beers.

It will also be seen from Fig. 2 that there is little, if any, change in the amounts of these *iso*humulones as the result of acid washing. On the other hand, the original *iso*-octane extract contained a group of substances emerging as some four peaks between 200 and 320 ml. of eluate. The greater part of these peaks is seen to be removed by the acid wash: these therefore appear to be the relevant group of components removed by the acid methanol wash in the Rigby & Bethune Method II.

Further support to this view is given by another set of Dr. Goedkoop's results shown of these peaks, showing that there is little present in this beer of relevant compounds, apart from the *iso*humulones themselves.

These results offer strong support to the Rigby & Bethune contention that their Method II gives an accurate method of measuring *iso*humulones as such, because the washed extract (Fig. 2) is seen to be a much purer solution of *iso*humulones. However, the original objective was to measure, not the *iso*humulone content but the bitterness of beer and it is necessary to retain this firmly in mind.

Dr. Goedkoop has shown further that the

compounds in the 200–320 ml. eluate group are bitter. Therefore, if both the "200–320 ml." compounds and the *iso*humulones are bitter and if both groups are extracted and measured in the Moltke-Meilgaard method, we are given a sound reason for using this method, especially if there is the possibility that the hops used may have undergone some deterioration.

It is interesting to speculate further on the nature of the compounds in the "200–320 ml. eluate." As mentioned earlier, Spetsig has shown that, when the α and β acids in older hops are diminished, the hulupones are increased. In addition in Dr. Goedkoop's work the absorption characteristics of the compound eluting with a peak at 315 ml. (λ max. in alkaline methanol 255 and 325 mμ.; λ max. in acid methanol 280 mμ.) correspond with those of a hulupone, while some of the substances in other peaks show characteristics which suggest they may have arisen by oxidation of humulones.

These facts therefore appear to draw together and co-ordinate the various points discussed throughout this paper.

The picture suggested is that in fresh hops almost the sole bittering compounds are the α acids which are the origin of the *iso*-humulones in the beer. These *iso*humulones can now be measured in the beer by the Rigby & Bethune II method or by the Moltke & Meilgaard method (or its variants) and the results, stated in I.B.U.s, will agree.

In old or deteriorated hops, while α acids are almost always still the predominant bittering compounds, there are significant amounts of hulupones and other preformed, water-soluble bitter compounds. These pass unaltered to the beer and contribute some bitterness in addition to that given by the *iso*humulones from the α acids. In such beers the Rigby & Bethune II method measures only the *iso*humulones, while the Moltke & Meilgaard or related methods include other bittering substances of the resin type, so giving a better measure of total bitterness.