was equivalent to 20.1 lb of fixed oil per ton of seed.

The meal residue used for the input fixed oil analysis consisted essentially of the whole seed with only two seed constituents removed besides the foreign matter: 3.3% as moisture and 19.0% as oil. Approximately 77% of the wt of the original seed including all of the ammonia or protein was present in the residue for input fixed oil analysis.

The cottonseed meal used for fixed oil analysis of the output had more seed constituents removed in the form of moisture, linters, hulls, a small protein fraction and oil leaving only approximately 47% by wt of the original seed for output fixed oil analysis.

The fatty acids from the fixed lipids of the two meals are directly proportional to their protein contents. Shuey et al. found a similar relationship in their work on the oil-binding capacity of wheat flour.

The quality of the fixed oil fatty acids was examined. Samples of residual oil in cottonseed meal representing ca 4% of the wt of the meal were obtained by petroleum ether extraction. The residual oil was hydrolyzed, methylated and the fatty acid composition determined by gas chromatography. The fatty acids derived from the fixed oil in the same meals were also methylated and their composition determined by gas chromatography. The two were essentially the same. The neutralization equivalents of the fatty acids from the fixed oil were determined by very slow titration in a carbon dioxide free atmosphere to give values approaching 278 and in the range observed for fatty acids from cottonseed oil.

When tested for cyclopropenoid fatty acids by the Halphen test (10) the fatty acids from the fixed oil fraction of screw press cottonseed meal gave a value of 0.15%, lower than is usually found in cottonseed oil. On the other hand, a sample of fatty acids derived from the fixed oil of the whole seed residues prepared in the laboratory (9) gave a value of 0.36%, which is about normal for cottonseed oil.

In their work on the processing of cottonseed meats Eaves et al. (11) found that during solvent extraction of raw cottonseed meats phosphorus-containing compounds are extracted with the oil in significant amts. They also found that cooking of the meats hindered the extraction of these phosphorus compounds. It would appear therefore that apart from an insignificant fraction most of the phospholipids present in the cottonseed are fixed in the meal as a result of cooking of the cottonseed meats when processed in an oil mill. The phospholipids present in the meal after petroleum ether extraction (7,9) also contribute their share to the fatty acids in the fixed oil determination.

The greatest significance of the fixed oil fraction is that any material balance for oil in an oil mill when made without taking into account the fixed oil fraction present in input and output meals is necessarily incomplete. A superior and more accurate material balance for oil may be obtained provided the results from determinations for fixed oil are used to supplement the results of analyses for oil by the Official AOCS Method (7,9). A valid material balance for oil should include the significant item of milling loss of oil which is not even recognized in the present state of affairs.

The Halphen-positive cyclopropenoid fatty acid constituents of the fixed oil are also of practical interest in expanding the use of cottonseed meal for poultry rations. Satisfactory quantitative estimation of the levels of these compounds present in given cottonseed meals can be made only by determination and study of the neglected, unknown fixed oil quantity in addition to the known and readily obtainable total oil in seed and residual oil in meals.

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• *Letters to the Editor*

Gas Chromatographic Analysis of Tall Oil Rosin Acids

A RAPID ANALYTICAL method which would provide a reasonably quantitative measure of rosin acid isomers was desired for our continuing study of rosin composition. Historically, methods of isomer analysis have been very time-consuming. Recently the gas chromatography of rosin acid methyl esters (1,2), prepared with diazomethane (3), has allowed rapid analysis. A drawback to this method lies in the hazards associated with diazomethane. Other methods of esterification, using acid catalysis, are excluded since the sterically hindered rosin acids esterify slowly and the labile double bond systems rearrange rapidly. A potential chromatographic method for rosin acids was suggested by a paper of Robb and Westbrook (4) who prepared methyl esters of fatty acids by decomposition of their tetramethylammonium salts.

Our work on the pyrolysis of the tetramethylammonium salts of rosin acids to methyl esters demonstrates that this approach can be used to provide a rapid, safe and accurate method for the determination of rosin acid isomer content. Tetramethylammonium salts are prepared by titration. Pyrolysis of these salts in the inlet port of the gas chromatograph conveniently forms the methyl esters which then separate on the chromatographic column.

Tetramethylammonium chloride (5) 2.7 g (24.6 mmoles) was dissolved in about 30 ml of anhydrous methanol. Silver oxide 5.8 g (25.1 mmoles) was added and the mixture stirred under anhydrous

TABLE I

Comparative Results
Diazomethane and Tetramethylammonium Salt Procedures[a]

Replication Inlet Temp.		Diazomethane Procedure				Tetramethylammonium Salt Procedure			
		1 400C[c]	2 320C	3 300C	Average 3 Runs	1 400C	2 405C	3 405C	Average 3 Runs
Peak No.	RRT[b]	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %
1	0.34	1.20	1.10	0.98	1.10	1.29	1.20	0.90	1.13
2	0.46	3.61	3.50	3.16	3.42	3.76	3.60	3.17	3.51
3 Pimarate	0.48	4.02	4.13	3.41	3.85	4.66	4.33	3.88	4.29
4	0.51	1.03	1.58	0.63	1.08	0.59	0.86	0.89	0.78
5	0.55	3.47	4.42	4.05	3.98	4.56	4.05	4.24	4.28
6 Palustrate	0.65	7.96	8.52	9.35	8.61	7.87	7.63	8.06	7.85
7	0.70	5.51	4.38	4.99	4.96	4.45	4.61	4.36	4.47
8	0.75	4.19	2.96	3.25	3.47	2.95	3.05	2.94	2.98
9	0.80	1.03	1.65	1.42	1.37	1.32	0.99	1.39	1.23
10 Abietate	1.00	27.79	30.23	31.50	29.84	32.23	33.23	33.31	32.92
11 Dehydro-abietate	1.08	32.09	27.57	28.29	29.32	31.92	30.86	30.84	31.21
12	1.15	2.06	3.18	2.51	2.58	3.13	3.65	4.65	3.81
13	1.25	6.02	6.77	6.47	6.42	1.25	1.99	1.38	1.54

[a] Chromatographic Conditions:

Instrument	F & M 500
Column	3/16 in. × 3M LAC 3R-728, 4% on 60-80 mesh Chromsorb G diatomaceous earth
Column temp	210C
Inlet temp	400C
Detector temp	245C
Inlet pressure	30 psig (helium)
Column flow rate	60 ml/min
Reference flow rate	40 " "
Bridge current	210 ma
Recorder sensitivity	1 mv full scale
Chart speed	40 in./hr

[b] RRT—Retention time relative to methyl abietate.
[c] Normally methyl esters prepared with diazomethane use an inlet temperature 300-320C.

conditions for 16 hr. The precipitated salts were removed by filtration and an aliquot of the filtrate titrated to a phenolphthalein endpoint with 0.1 *N* hydrochloric acid. The remainder of the filtrate (0.58 meq/ml) was diluted with sufficient dry benzene to make a 0.1 *N* tetramethylammonium hydroxide solution. Molar tetramethylammonium hydroxide solution may be obtained from Southwestern Chemicals Company, Austin, Texas.

A sample of a typical tall oil rosin acid, 0.1 g, was dissolved in 1 ml of methanol. One drop of phenolphthalein solution was added and the mixture titrated with the 0.1 *N* tetramethylammonium hydroxide solution until a pink endpoint was obtained. A portion of the tetramethylammonium salt solution was then used for injection into the gas chromatograph.

Methyl esters were prepared with diazomethane by the method of Schlenk and Gellerman (3).

Samples of the tetramethylammonium salt solutions, ranging in size from 10 to 40μl, were injected into the instrument. The salts pyrolyzed and the resultant methyl esters passed through the column. Complete elution required about 45 min. The peak areas were obtained by multiplication of peak height and width at one-half peak height.

Comparison of chromatographic values obtained on a sample of tall oil rosin acids using the diazomethane and the tetramethylammonium salt pyrolysis methods are shown in Table I. Reproducibility of results employing the tetramethylammonium salt method is shown in Table II. The replications were obtained over a period of several days to demonstrate day to day reproducibility. The satisfactory agreement between these methods and the good reproducibility of the tetramethylammonium salt procedure is evident. The divergent values obtained for peak 13 was the only significant difference noted.

The recovery of methyl esters of rosin acids by the two methods was investigated. Aliquots of a tall oil rosin sample, rich in dehydroabietic acid and with dibutyl sebacate added as an internal standard, were carried through both procedures. There was no significant difference in the amount of methyl dehydroabietate recovered as measured by the area ratio of ester to the standard.

TABLE II

Reproducibility
Tetramethylammonium Salt Pyrolysis Procedure

Peak Number	Tentative Identification Methyl Ester of:	Rel. Std. Deviation
3	Pimaric acid	9.1%
6	Palustric acid	2.7
10	Abietic acid	1.8
11	Dehydroabietic acid	2.0

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