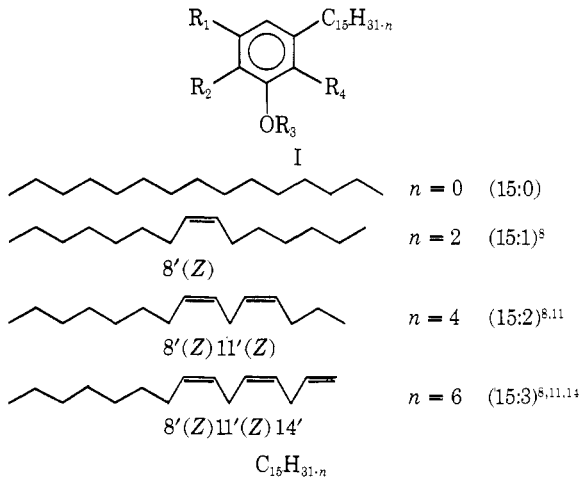
Determination of the Component Phenols in Natural and Technical Cashew Nut-Shell Liquid by Gas–Liquid Chromatography

John H. Tyman

School of Chemistry, Brunel University, Uxbridge, Middlesex, England

The component phenols in the industrially useful technical cashew nut-shell liquid and in the natural product have until now only been estimated. By the use of gas–liquid chromatographic methods, they have been determined with much improved precision. The main difficulties in the analysis lie in the widely differing proportions of the component phenols which exist in different levels of unsaturation, and in overcoming their nonvolatility. By the use of the methyl ethers of the hydrogenated technical and natural products which can be quantitatively obtained, symmetrical peaks were obtained which enabled standard integration procedures to be applied and the component phenols to be quantitatively determined. The method is quicker to operate than any preceding method. On polyethylene glycol adipate columns, the mean results (with standard deviations) for the C_{15} phenols in natural cashew nut-shell liquid were 82.0 ± 1.05% anacardic acid, 13.8 ± 0.79% cardol, 2.6 ± 0.16% 2-methylcardol and 1.6 ± 0.17% cardanol. For technical cashew nut-shell liquid, the mean results were 83.0 ± 0.51% cardanol, 14.3 ± 0.58% cardol, 2.7 ± 0.34% 2-methylcardol.

The principal component phenols in cashew nut-shell liquid (CNSL) from *Anacardium occidentale* are anacardic acid (I, $R_1 = R_2 = R_3 = H$, $R_4 = CO_2H$, $n = 0, 2, 4, 6$) (*1–3*), cardol (I, $R_2 = R_3 = R_4 = H$, $R_1 = OH$, $n = 0, 2, 4, 6$) (*4*), 2-methylcardol (I, $R_3 = R_4 = H$, $R_1 = OH$, $R_2 = CH_3$, $n = 0, 2, 4, 6$) (*5, 6*), and cardanol (I, $R_1 = R_2 = R_3 = R_4 = H$, $n = 0, 2, 4, 6$) (*7*).



The natural product is thermally decarboxylated to yield the industrially useful (*8, 9*) "technical CNSL", containing mainly cardanol. The initial column chromatographic separations of cardanol methyl ether from methylated technical CNSL on alumina and its subsequent resolution on a more active column into the saturated, monoene, diene, and triene constituents were described by Symes and Dawson (*7*). Technical CNSL has been separated on silica gel (*8*) and also natural CNSL (*9*) after removal of anacardic acid by the lead salt procedure and by preliminary passage through alumina (*6*). Column chromatographic methods have, however, tended to be estimations rather than determinations of the phenolic composition. Other physical techniques used have been argentation TLC (*3*) and, less effectively, low temperature crystallization (*2*).

An adsorption thin layer chromatographic/uv spectrophotometric method was effective for determination of the phenolic composition of natural CNSL (*5*), although technical CNSL caused difficulties.

A gas–liquid chromatographic determination with the stationary phase SE-30 has been employed for technical and natural CNSL after hydrogenation of the unsaturated side chains and conversion of (15:0) anacardic acid to the volatile methyl ester (*10*). Insufficient resolution of (15:0) cardol and 2-methylcardol and skewed peaks has led to the desirability of converting all the (15:0) phenols to volatile derivatives. A variety of these have been examined and this present account is concerned with the (15:0) methyl ethers (I, $R_1 = H$ or OCH_3, $R_4 = H$ or CO_2CH_3) chromatographed on the more polar stationary phases polyethylene glycol adipate (PEGA) and Dexsil 300 which have led to an improved analytical procedure with respect to resolution and formation of symmetrical peaks. In the absence of the hydrogenation step to convert the unsaturated constituents to the (15:0) member, PEGA also provides the best means for determination of the olefinic constituents after preliminary TLC separation of the component phenols (*11*).

EXPERIMENTAL

Preparation of Materials. Natural CNSL was extracted as described (*6, 10, 11*) and technical CNSL obtained as before. Saturated natural and technical CNSL were obtained by hydrogenation as described (*6, 10*). Completion of hydrogenation was followed by the disappearance (scale expansion necessary) of olefinic signals in the ^1H NMR absorption spectrum. The anacardic acid in saturated natural CNSL was first esterified (*10*) in ethereal solution at 0°C with ethereal diazomethane (to avoid any decarboxylation). The phenolic groups were then methylated with dimethyl sulfate in refluxing benzene solution containing anhydrous potassium carbonate as described (*10, 11*) with the modification that a 10 *M* proportion was used and completion of methylation monitored by GLC (on an SE-30 column to detect phenols), TLC and infrared spectrometry (disappearance of OH absorption). Saturated technical CNSL was methylated directly by the dimethyl sulfate method.

(15:0) Cardanol, cardol, and 2-methylcardol were obtained as described (*6, 11*) and methylated with dimethyl sulfate in hot benzene containing anhydrous potassium carbonate. (15:0) Cardanol methyl ether crystallized as white prisms from light petroleum, mp 28–29 °C (lit. (*12*) 29–30 °C), (15:0) cardol dimethyl ether from light petroleum–benzene as white prisms, mp 45.5–46.5 °C (lit. (*12*) 48.5–49 °C) and (15:0) 2-methylcardol dimethyl ether obtained (*6*), mp 39–40 °C.

The methyl ester methyl ether of (15:0) anacardic acid was obtained by the successive diazomethane, dimethyl sulfate procedure as white plates, mp 39–40 °C (lit. (*11, 12*) 37–37.5 °C).

To obtain anacardic free from cardol in a modified lead salt separation, it was important to thoroughly stir the precipitated and

filtered lead anacardate with ethanol and refilter prior to liberation of the anacardic by acidification in the cold (0 °C) with dilute nitric acid.

Gas–Liquid Chromatography. The gas chromatographic equipment used was as described (*10, 11*). The column support, diatomite C was acid-washed and silanized and used with the mesh range indicated; 3% SE-30 (100–120 BSS), 2% PEGA (80–100) and 3% Dexsil 300 (80–100). A flow-rate of 45 ml/min of nitrogen (equal to an inlet pressure of 8 psi) was used. The pre-heater was approximately 50 °C greater than the column temperature. A chart speed of 0.5 cm/min was generally used. For integrations, considerably higher speeds were desirable. Repeated attenuation changes complicated the integration procedure and triangulation was invariably used.

Both triangulation and integration were carried out and agreed closely (*10*). The accurate drawing of triangles was facilitated by the use of a powerful lens supported over the chart paper. To obtain peaks of similar general size, attenuation adjustment was used throughout. An alternative method used only occasionally was to obtain the major peaks on the chart paper by means of a small sample injection and the minor peaks considerably increased by the use of a tenfold sample. A common peak was used to interrelate the results from the two runs. Unavoidably, some peaks just exceeded the width of the chart paper and, in such cases where the analysis was a lengthy procedure, the band was a squat triangle and the distances were short, the peak was constructed on an attached strip of paper. Complete agreement of results with normal (on chart) peaks was obtained and justified this very occasional expedient.

Results by triangulation, with and without attenuation adjustment, agreed within 0.2%. The normalized peak areas from triangulation were the result of at least six determinations in each case. Standard deviations (S) were calculated.

Thin layer chromatography was carried out as described (*10*).

Calibration Experiments. The flame ionization detector was calibrated both for relative response factors of the phenolic methyl ethers and for its linearity. The latter has been well established for other substances over a wide concentration range (*13, 14*). Linearity was established over the concentration range in the present work from accurate dilutions of a standard solution and a plot of log (peak area)/log (sample weight) (*15*).

Because of the slight overlap of the (15:0) cardol dimethyl ether and (15:0) 2-methylcardol dimethyl ether peaks, two standard solutions were prepared, the first containing the dimethyl derivative of (15:0) anacardic acid, the dimethyl ether of (15:0) cardol, and the methyl ether of (15:0) cardanol and the second containing the methyl ether of (15:0) cardanol and the dimethyl ether of (15:0) 2-methylcardol. The results from the two series were then interrelated. Materials were weighed on a five-place balance and solutions made up in 2-ml graduated flasks in benzene solution.

RESULTS AND DISCUSSION

Relative Retentions of Derivatives of the Component Phenols on Different Stationary Phases. The retention times (RT, in min) and relative retentions (RR) of the methyl ethers of (15:0) component phenols on SE-30, PEGA, and Dexsil columns are given in Table I (Figures 1, 2, and 3). The relative retentions of the phenols with respect to the corresponding methyl ethers were prohibitively high except on SE-30 but the skewed peaks which tended to result made derivative examination desirable. The coincident retention times for dimethyl (15:0) anacardate and the dimethyl ether of (15:0) 2-methylcardol precluded the use of this stationary phase. Increase in polarity from SE-30, PEGA to Dexsil 300 resulted in a great increase in the relative retention. With Carbowax 20M at the temperature limit recommended (180 °C), the retention times became exceedingly long with consequent broadening of the peaks. On PEGA, the dimethyl ether of (15:0) 2-methylcardol emerged before that of (15:0) cardol whereas on Dexsil the order was reversed although the 2-methylcardol derivative was eluted just sufficiently before dimethyl (15:0) anacardate to permit analysis.

The degree of separation of the (15:0) 2-methylcardol and cardol derivatives on PEGA was important for the accuracy of the method. In early work on a rather inefficient

Table I. Retention Times (RT) and Relative Retentions (RR) of the Methyl Ethers of the Component Phenols of CNSL

Column	Temp, °C	(15:0) Cardanol (C_{15}) methyl ether			(17:0) Cardanol (C_{17}) methyl ether			(15:0) Cardol dimethyl ether			(15:0) 2-Methylcardol dimethyl ether			(15:0) Dimethyl anacardate		
		RR^a	RT	RR^b	RR	RT	RR	RR	RT	RR	RR	RT	RR	RR	RT	RR
2% PEGA	180	(1)	50.4	(2.18)	(1.87)	92.4	(4.00)	(3.23)	163.2	(7.06)	(2.91)	146.9	(6.36)	(5.53)	279.2	(12.09)
2% PEGA	190	(1)	32.2	(1.39)	(1.84)	59.2	(2.56)	(3.11)	100.2	(4.34)	(2.81)	90.4	(3.91)	(5.16)	166.0	(7.19)
2% PEGA	200	(1)	23.1	(1)	(1.73)	40.0	(1.73)	(2.83)	65.3	(2.83)	(2.56)	59.1	(2.56)	(4.59)	106.1	(4.59)
2% PEGA	220	(1)	12.0	(0.52)		...		(2.64)	31.9	(1.38)	(2.28)	27.4	(1.19)	(3.88)	46.8	(2.03)
3% Dexsil 300	220	(1)	49.8	(2.16)		...		(2.15)	107.2	(4.64)	(2.47)	123.2	(5.34)	(2.73)	136.0	(5.89)
3% Dexsil 300	230	(1)	34.7	(1.51)		...		(2.07)	72.0	(3.12)	(2.36)	82.1	(3.56)	(2.59)	90.2	(3.91)
3% Dexsil 300	250	(1)	18.1	(0.78)		...		(1.90)	34.4	(1.49)	(2.12)	38.4	(1.67)	(2.33)	42.2	(1.83)
3% Dexsil 300	290	(1)	6.8	(0.28)		...		(1.63)	11.1	(0.48)		...[c]		(1.91)	13	(0.57)
3% SE-30	220	(1)	12.8	(.56)		...		(1.89)	24.2	(1.05)	(2.12)	27.2	(1.18)	(2.32)	29.85	(1.29)

[a] Relative retention with respect to (15:0) cardanol methyl ether at that particular temperature (1st parentheses). [b] Relative retention with respect to (15:0) cardanol methyl ether at 200 °C (2nd parentheses). Retention distances measured to center of peak. [c] Not resolved from the cardol dimethyl ether peak.

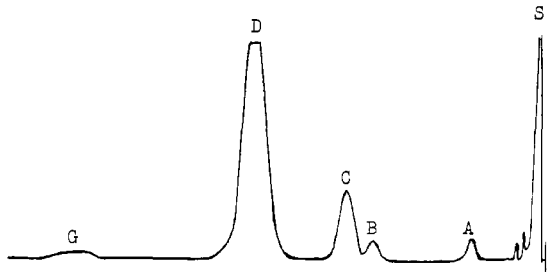


Figure 1. Gas–liquid chromatogram of natural CNSL on 2% PEGA (Figures 1 and 3 shown at half the normal chart speed)

Figure 2. Natural CNSL on 3% Dexsil

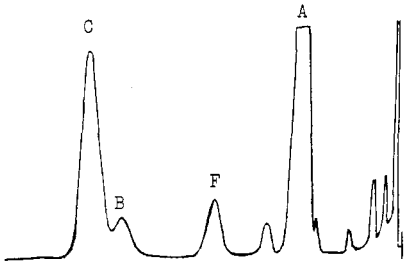


Figure 3. Technical CNSL on 2% PEGA

(A) (15:0) Cardanol methyl ether. (B) (15:0) 2-Methylcardol dimethyl ether. (C) (15:0) Cardol dimethyl ether. (D) (15:0) Dimethyl anacardate. (F) (17:0) Cardanol methyl ether. (G) (17:0) Dimethyl anacardate

column having approximately 800 plates, the resolution was 0.76 while on a column with 1000 plates the resolution was 1.16 and accurate triangulation was possible. Generally, although with PEGA the stationary phase was being employed in the region of the temperature limit sometimes recommended for this column, the results were more satisfactory than with Dexsil 300 since all the peaks were clearly resolved. With Dexsil 300, it had been hoped that the considerably higher temperature limit of 350 °C together with greater polarity would have permitted rapid analysis. However, at 290 °C, the (15:0) 2-methylcardol and cardol derivatives were not resolved and a considerably lower temperature (220 °C) with inconveniently long retention times proved to be mandatory. The greater thermal stability of Dexsil 300 might have enabled the phenols rather than the methyl ethers to be used but pronounced tailing precluded this.

Calibration of the Flame Ionization Detector towards Methyl Derivatives of the Saturated Component Phenols. The percentages (and standard deviations) from peak areas of component methyl ethers, the average areas, the weights and response factors obtained by dividing the average peak area by the weight of the particular component have been summarized in Tables II and III. Interrelationship of these two series gave the relative response factors of 0.8754 (dimethyl (15:0) anacardate), 0.8341 (15:0) 2-methylcardol dimethyl ether, and 0.8822 (15:0) cardol dimethyl ether with reference to (15:0) cardanol methyl ether (1.0000). It is noteworthy that cardanol methyl ether has the highest value like cardanol in the phenol series.

Composition of the Component Phenols in Natural and Technical CNSL. Table IV shows the uncorrected % composition (and standard deviation) from the normalized peak areas of the four (15:0) phenolic methyl ethers, the relative response factors, the corrected % composition for the (15:0) methyl ethers, obtained by dividing the peak area by the respective relative response factor, the corrected % composition for the (15:0) phenols and for the unsaturated phenols. The last line in the table has been calculated on the basis that natural cardanol and anacardic acid are dienoid in average composition while cardol and 2-methylcardol are primarily trienoid (*10*).

On Dexsil 300 (15:0), 2-methylcardol dimethyl ether appeared between (15:0) dimethyl anacardate and (15:0) cardol dimethyl ether and was not quite resolved from the for-

Table II. Response Factors for (15:0) Cardanol Methyl Ether, (15:0) Cardol Dimethyl Ether, and Dimethyl (15:0) Anacardate

	(15:0) Cardanol methyl ether	(15:0) Cardol dimethyl ether	Dimethyl (15:0) anacardate
% component from normalized peak areas	31.35 ± 0.980	29.95 ± 0.318	38.68 ± 0.828
Av peak area/10^5	0.36176	0.33658	0.43208
Wt taken, g	0.05511	0.05812	0.07519
Response factor	6.5643	5.7911	5.7465

Table III. Response Factors for (15:0) Cardanol Methyl Ether and (15:0) 2-Methylcardol Dimethyl Ether

	(15:0) Cardanol methyl ether	(15:0) 2-Methylcardol dimethyl ether
% component from normalized peak areas	76.28 ± 0.828	23.72 ± 0.650
Av peak area/10^5	0.31243	0.09756
Wt taken, g	0.03617	0.01354
Response factor	8.6378	7.2054

Table IV. Composition of the C_{15} Component Phenols of Natural CNSL

	Anarcardic acid	Cardol	2-Methylcardol	Cardanol
Uncorrected normalized % from peak areas of (15:0) phenol methyl ether	81.68 ± 1.05	14.03 ± 0.789	2.53 ± 0.163	1.76 ± 0.174
Relative response factors	0.8754	0.8822	0.8341	1.0000
Corrected normalized % (15:0) methyl ether	81.84	13.95	2.66	1.54
Corrected normalized % (15:0) phenols	81.88	13.87	2.65	1.59
Corrected normalized % (unsaturated phenols)	81.98	13.78	2.64	1.59

Table V. Composition of the C_{15} Component Phenols of Technical CNSL

	Cardol	2-Methylcardol	Cardanol
Uncorrected normalized % from peak areas of (15:0) phenol methyl ether	13.43 ± 0.582	2.35 ± 0.336	84.22 ± 0.514
Relative response factors	0.8822	0.8341	1.0000
Corrected normalized % (15:0) phenol methyl ether	14.88	2.76	82.36
Corrected normalized % (15:0) phenols	14.41	2.68	82.91
Corrected normalized % (unsaturated phenols)	14.34	2.67	82.99

Table VI. Distribution of Cardanol Homologues with Relative Retentions

No. of C atoms	% component/% C_{15} (15:0)	Relative retention distances (*RD*)	log (*RD*)
9	0.46	1.84	0.264
11	0.61	3.27	0.515
13	0.39	5.93	0.773
15	100	10.54	1.022
17	3.15	18.85	1.275

(13.78%) cardol, (2.64%) 2-methyl cardol, and (1.59%) cardanol the theoretical decarboxylated product would contain (81.67%) cardanol, (15.40%) cardol and (2.94%) 2-methylcardol. The actual technical CNSL examined contained (82.98%) cardanol, (14.34%) cardol, and (2.67%) 2-methylcardol. The difference can be accounted for by the partial polymerization resulting from the thermal decarboxylation process.

TLC examination has shown that 20% of technical CNSL is polymerized and nonvolatile, and the remainder is the volatile fraction to which the GLC results relate. It can be readily calculated that the 20% of polymerized material contains 76.35% of polymerized cardanol, 19.65% of polymerized cardol and 4.22% polymerized 2-methylcardol and the disappearance of relatively more cardol than cardanol occurs.

Table VII. Composition of Cardanol (C_{15}, C_{17}) in Technical CNSL

			Cardanol	
	Cardol	2-Methylcardol	C_{17}	C_{15}
Uncorrected normalized % from peak areas of (15:0) phenol Me ether	13.05	2.26	2.58	82.11
Relative response factors	0.8822	0.8341	1.0000	1.0000
Corrected normalized %	14.471	2.653	2.531	80.346
Corrected normalized % (15:0) phenols	14.03	2.58	2.56	81.00

mer. The result of a single analysis by injection of a sample followed by a tenfold sample in order to obtain greater accuracy in the analysis of the two minor components gave the following uncorrected normalized results, (82.10%) (15:0) dimethyl anacardate, (14.12%) (15:0) cardol dimethyl ether, (2.24%) (15:0) 2-methylcardol dimethyl ether, and (1.74%) (15:0) cardanol methyl ether which can be compared with the first line in Table IV and, with the exception of the 2-methyl cardol result, show a close similarity to the results on PEGA. Similarly the composition of technical CNSL is given in Table V.

The results for natural CNSL on PEGA columns show a higher % anacardic acid than in the preliminary series (*11*) carried out with the phenols on an SE-30 column, namely (77.14%) anacardic acid, (16.67%) cardol, (3.81%) 2-methylcardol, and (2.37%) cardanol.

The discrepancy may be ascribed to errors in the determination of peak areas of asymmetrical peaks and to chemical errors from possible slightly incomplete conversion of anacardic acid to the methyl ether by means of diazomethane. The methylation procedure with dimethyl sulfate could be achieved quantitatively and traces of anacardic acid remaining were converted to the dimethyl derivative.

In the case of technical CNSL, the results show closer agreement with those obtained for the phenols on SE-30, namely (82.22%) cardanol, (13.71%) cardol, and (4.15%) 2-methylcardol and emphasize that the discrepancy in the case of natural CNSL may be largely chemical in origin. Additionally, in the analysis of technical CNSL on SE-30, despite the silanization of the column and its support, some adsorption may lead to a small error.

It was not possible to obtain the natural CNSL used specifically for the preparation of the technical CNSL although both originated in Mozambique. From natural CNSL having the composition (81.98%) anacardic acid,

The methyl ethers of the major components of natural CNSL had been identified by comparison of their retention distances with synthetic materials. The presence of a C_{17} anacardic acid in natural CNSL (*6*) and correspondingly a C_{17} cardanol in technical CNSL had been indicated in this way. In the latter material, partly because they were among the first eluted peaks in the chromatogram and not obscured by other peaks as in the case of homologous anacardates, C_{13}, C_{11}, and C_9 homologues were detected. The estimated proportions together with their retention distances have been summarized in Table VI. A linear plot was obtained from log (retention distance)/chain length. The presence of C_{13}, and C_{17} homologues in *Anacardium occidentale* has been reported during a particular GLC study of the total acidic composition (*16*), with the rather inaccessible stationary phase β-cyclodextrin acetate. C_{11} saturated and C_{17} unsaturated anacardic acids have been found in *Anacardium giganteum* (*17*), *Pentaspadon motleyi* (*18*), and *Pentaspadon officianalis* (*19*), respectively. In the case of technical CNSL, a few minor peaks remained unidentified by the comparison procedure with synthetic reference compounds. Since the chromatogram of natural CNSL is simpler, the unknown peaks may possibly arise as a result of the thermal decarboxylation process. Their identity is currently being investigated by mass spectrometry/GLC.

If the analytical results for technical CNSL are recalculated to include C_{17} cardanol in the total analysis, the fig-

ures shown in Table VII are obtained (C_{13}, C_{11}, C_9 cardanols have not been taken into account). The total derivable cardanol (83.56%) is slightly higher than if only the C_{15} component had been considered. Expressed in terms of the same % cardol (14.34%), the total (C_{15} and C_{17}) cardanol is 85.41%.

Pillay (*20*) thought that "a thorough diagnosis of CNSL by all known instrumental methods is still to come". The stationary phase PEGA used in the present method for the component phenols and also in the TLC/GLC method for the unsaturated constituents (*13*) provides a general method for the total analysis of CNSL and for the study of component phenols and their unsaturated constituents in other phenolic species.

LITERATURE CITED

(1) P. T. Izzo and C. R. Dawson, *J. Org. Chem.*, **14**, 1039 (1949).
(2) V. J. Paul and L. M. Yeddanapali, *J. Am. Chem. Soc.*, **78**, 5675 (1956).
(3) J. H. P. Tyman and N. Jacobs, *J. Chromatogr.*, **54**, 83 (1971).
(4) W. F. Symes and C. R. Dawson, *Nature*, **171**, 841 (1953).
(5) J. H. P. Tyman and L. J. Morris, *J. Chromatogr.*, **27**, 287 (1967).
(6) J. H. P. Tyman, *J. Chem. Soc., Perkin Trans. 1*, 1639 (1973).
(7) W. F. Symes and C. R. Dawson, *J. Am. Chem. Soc.*, **75**, 4952 (1953).
(8) B. G. K. Murthy, M. A. Siva Samban, and J. S. Aggarwal, *J. Chromatogr.*, **32**, 519 (1968).
(9) J. H. P. Tyman and C. A. Barrett, Brunel University, Batchelor Dissertation, 1971.
(10) J. H. P. Tyman, *J. Chromatogr.*, **111**, 277 (1975).
(11) J. H. P. Tyman, *J. Chromatogr.*, **111**, 285 (1975).
(12) H. J. Backer and N. H. Haack, *Rec. Trav. Chim. Pays-Bas*, **60**, 661 (1941).
(13) I. A. Fowlis, R. J. Maggs, and R. P. W. Scott, *J. Chromatogr.*, **15**, 471 (1964).
(14) R. J. Maggs and W. G. Pye, *Gas Chromatogr. Bull.*, **1**, (2), 2 (1966).
(15) H. M. McNair and E. J. Bonelli, "Basic Gas Chromatography", Varian Aerograph, Walnut Creek, Calif., 1965, p 89.
(16) J. L. Gellerman and H. Schlenk, *Anal. Chem.*, **40**, 739 (1968).
(17) N. K. Sharma and V. N. Sharma, *Indian J. Chem.*, **4**, 320 (1966).
(18) H. J. Backer and N. H. Haack, *Rec. Trav. Chim. Pays-Bas*, **60**, 678 (1941).
(19) J. A. Lamberton, *Aust. J. Chem.*, **12**, 234 (1959).
(20) P. P. Pillay, "Cashew Nut shell liquid Patents", Cashew Export Promotion Council, Ernakulam—6, India, Vol. 1, (introduction), 1964, p 3.

RECEIVED for review May 12, 1975. Accepted September 15, 1975.

Gas–Liquid Chromatographic Determination of Cocaine and Benzoylecgonine in Urine

Jack E. Wallace,* Horace E. Hamilton, David E. King, Diana J. Bason, Harvey A. Schwertner, and Steven C. Harris

Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Cocaine and its principal metabolite, benzoylecgonine (BE), are determined by gas–liquid chromatography (3% OV-17, FID) following extraction from urine into chloroform–ethanol (80/20, v/v) and methylation of BE to cocaine. The recovery from biologic specimens of 93 and 65% for cocaine and BE, respectively, and 72% conversion of BE to cocaine provide detection limits of <0.1 and 0.2 μg/ml for cocaine and BE, respectively. Separate simultaneous determinations of cocaine and benzoylecgonine are achieved by analyzing both a methylated (combined cocaine and BE) and nonmethylated (cocaine only) aliquot of the specimen extract. Data on drug levels in urine specimens from post-surgery patients receiving cocaine anesthesia are presented.

The illicit use of cocaine (*1–7*) has prompted considerable interest in the development of methods for the detection of users and abusers of the drug. Thin-layer chromatography (TLC) and EMIT (enzyme-multiplied immunoassay technique) are currently the most frequently employed screening methods for the detection of cocaine (*2*); however, these methods are inherently only semiquantitative at best. Wallace et al. (*8*), in a recent report that described a sensitive TLC procedure for determining cocaine and benzoylecgonine in urine, reviewed various techniques applicable to the detection of the drug and its principal metabolite. Although cocaine may be determined by gas–liquid chromatography (GLC), the amount of unchanged cocaine excreted in the urine (*2, 8, 9*) is generally below the limits of detection by most currently available methods (*10, 11*). Blake et al. (*12*) have proposed a sensitive GLC procedure for the analysis of cocaine which involves reduction of the drug with lithium aluminum hydride, derivatization of the reduced product with pentafluoropropionic anhydride and subsequent detection by electron capture. The application of the technique to the quantitation of the drug in biologic specimens has not been established.

The analysis of cocaine in urine is essentially unrewarding considering the fact that only approximately 0.5 to 1.0% of the total amount of drug and metabolites excreted is unchanged cocaine. Benzoylecgonine, the primary metabolite, is of such high water solubility that it does not rapidly enter into any of the organic solvents employed in standard extraction procedures (*2, 13*). In fact Koontz et al. (*14*) refer to benzoylecgonine as an amino acid that is extracted from aqueous solutions, e.g. urine, by organic solvents poorly or not at all. Many of these previously considered insuperable problems concerning benzoylecgonine extraction from biologic specimens have been circumvented in the extraction technique of this report. In addition, the primary cocaine metabolite requires conversion to a specific volatile derivative before it will pass through the type of column normally employed in gas–liquid chromatography (*2, 14*). Koontz et al. (*14*) and Fish and Wilson (*15*) have developed GLC methods in which extracted benzoylecgonine is methylated to cocaine prior to GLC detection. The method of Koontz affords the detection of 1 μg/ml benzoylecgonine in 10 ml of urine, but requires an extensive preparatory TLC clean up and is nonquantitative. The method of Fish and Wilson provides for linear recoveries of benzoylecgonine from urine over the range 1–14 μg/ml, but requires a continuous liquid–liquid extraction. Quantitative data (percent recovery, standard deviation, or limit of detection) were not presented.

The procedure of the present report described a GLC procedure capable of determining both cocaine and benzoylecgonine combined or cocaine alone with quantitative