Estimation of 4-Butoxyphenylacetohydroxamic Acid Utilizing the Lossen Rearrangement

D. DELL, D. R. BOREHAM, and B. K. MARTIN

Abstract □ A sensitive and specific GLC method for the determination of 4-butoxyphenylacetohydroxamic acid (bufexamac) is described. The trimethylsilyl derivative of bufexamac is chromatographed with docosane as the internal standard. This derivative of bufexamac undergoes the Lossen rearrangement on the column to give a quantitative yield of 4-butoxybenzylisocyanate. Possible metabolites of the drug, 4-butoxyphenylacetic acid and 4-butoxyphenylacetamide, can be simultaneously estimated.

Keyphrases □ 4-Butoxyphenylacetohydroxamic acid—Lossen rearrangement, GLC analysis, plasma, urine □ Bufexamac—Lossen rearrangement, GLC analysis, plasma, urine □ Lossen rearrangement—4-butoxyphenylacetohydroxamic acid (bufexamac) □ GLC—analysis, bufexamac

Bufexamac (4-butoxyphenylacetohydroxamic acid[1]) was shown to possess anti-inflammatory properties (1–4). Lambelin *et al.* (5, 6) used the colorimetric method of Bergmann and Segal (7) to determine the hydroxamic acid function in the serum of patients dosed with bufexamac. This method lacks specificity because it detects not only bufexamac but also hydroxy derivatives (substitution in the aromatic ring and/or the butoxy side chain) of bufexamac. The lower limit of sensitivity was reported as 3 mcg.

The present paper describes a GLC method which utilizes the Lossen rearrangement and is capable of determining bufexamac independently of metabolites.

Esters of hydroxamic acids readily undergo a Lossen rearrangement (8) on heating to form the corresponding isocyanates, a reaction that was reviewed by Yale (9) and Franklin (10). By applying this method to bufexamac, the reaction in Scheme I may be written. The free acids also undergo this reaction but only very slowly and usually not quantitatively.

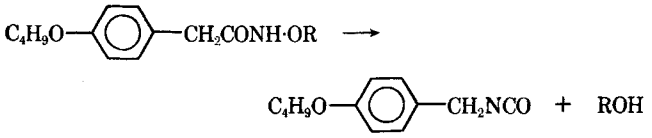
The method described involves the application of this rearrangement to the analysis of bufexamac in plasma and urine *via* the trimethylsilyl ether derivative.

EXPERIMENTAL

Reagents—All solvents used were of A.R. grade purity. Bufexamac dissolved in chloroform (114 mcg./ml.) was a stock solution for calibration, as was 4-butoxyphenylacetic acid in chloroform (99 mcg./ml.). The derivatization reagent was bistrimethylsilylacetamide[2] dissolved in tetrahydrofuran (1:2 v/v). The internal standard was docosane[3] dissolved in carbon tetrachloride (124 mcg./ml.).

Apparatus—A Philips PV-4000 gas chromatograph[4] with a flame-ionization detector was used. The column was a 3.0-m. × 4-mm. (i.d.) glass coil with internal walls silanized; it was packed with Gas Chrom Q[5] (80–100 mesh) and coated with 9% OV-17[6].

[1] Droxaryl, Continental Pharma S. A., Belgium.
[2] Phase Separations Ltd., Flintshire, Scotland.
[3] Koch-Light Labs. Ltd., Colnbrook, Bucks, England.
[4] Pye-Unicam Ltd., Cambridge, England.
[5] Applied Science Labs Inc., State College, PA 16801
[6] Perkin-Elmer Ltd., Beaconsfield, Bucks, England.



Scheme I

The column temperature was 220°; the flow rate was 40 ml. N_2/min. Under these conditions, the trimethylsilyl derivatives of bufexamac and 4-butoxyphenylacetic acid gave single peaks with retentions 0.356 and 0.516, respectively, relative to docosane.

Preparation of 4-Butoxybenzylisocyanate—4-Butoxyphenylacetohydroxamic acid (2.2 g.) was dissolved in tetrahydrofuran (140 ml.) containing pyridine (2 ml.); acetic anhydride (2 ml.) was added, and the solution was allowed to stand at room temperature for 10 min. The solution was concentrated *in vacuo* (15 mm. of mercury) and poured into cold water. The precipitated solid was collected, dried, and recrystallized from hot ethyl acetate–acetone (50:1 v/v) to give the acetyl derivative of bufexamac as colorless flakes, m.p. 160–161°.

Anal.—Calc. for $C_{14}H_{19}NO_4$: C, 63.6; H, 7.18; N, 5.29. Found: C, 63.7; H, 7.25; N, 5.18.

The acetyl derivative was heated at 180° at 0.5-mm. pressure to provide a colorless liquid as distillate, which was characterized as 4-butoxybenzylisocyanate by IR spectroscopy and elemental analysis.

Anal.—Calc. for $C_{12}H_{15}NO_2$: C, 70.2; H, 7.32; N, 6.83. Found: C, 69.7; H, 7.55; N, 6.53.

The IR spectrum showed strong absorption at 2270 $cm.^{-1}$, indicative of an isocyanate. This compound was unstable and changed very rapidly to a white solid.

METHODS

General Procedure—Various volumes of the standard chloroform solution of bufexamac, containing 1–50 mcg., were evaporated to dryness under reduced pressure (15 mm. of mercury) at room temperature in pear-shaped flasks. Tetrahydrofuran (100 μl.) was added, followed by bistrimethylsilylacetamide (50 μl.). The solution was allowed to stand at room temperature for 10 min. with occasional shaking. It was shown that silylation of bufexamac, under these conditions, is complete after 5 min. The internal standard solution (0.5 ml.) was then added, and 1 μl. of the final solution was injected onto the column. The ratios of peak heights (4-butoxybenzylisocyanate–docosane) were plotted against the amount of bufexamac.

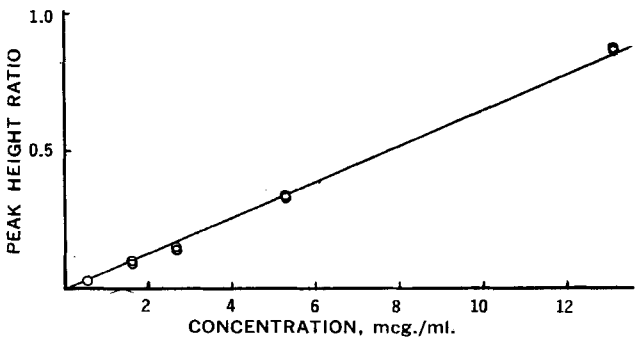


Figure 1—*Relationship between relative peak height and bufexamac concentration in urine.*

Table I—Recovery of Bufexamac from Urine and Plasma

Urine			Plasma		
Micrograms per Milliliter		Mean Percentage Recovery	Micrograms per Milliliter		Mean Percentage Recovery
Added	Found		Added	Found	
16.40	14.50, 14.40	88.1	16.40	13.70, 13.70	83.3
6.57	5.77, 5.60	86.5	6.57	5.60, 5.60	85.2
3.28	2.66, 2.54	79.3	3.28	2.71, 2.67	82.0
1.97	1.63, 1.72	84.9	1.97	1.50, 1.33	71.8
0.66	0.55, 0.58	86.0	0.66	0.54, 0.54	82.7
Overall recoveries		85.0 ± 3.4			81.0 ± 5.3

A similar procedure was adopted for 4-butoxyphenylacetic acid, using the standard choloroform solution.

Extraction from Urine—*Bufexamac*—Two aqueous solutions of bufexamac, containing 19.7 mcg./ml. (I) and 1.97 mcg./ml. (II), were used. From Solution I, 2.5-, 1.0-, and 0.5-ml. samples were taken in duplicate. From Solution II, 3.0- and 1.0-ml. samples were taken, also in duplicate. Each solution was mixed with urine (3 ml.) and 1 *N* HCl (1 ml.), and the volume was adjusted to 9 ml. by the addition of distilled water. Chloroform (25 ml.) was added, and the mixture shaken mechanically for 10 min. An aliquot (20 ml.) of the chloroform extract was evaporated and treated as described previously to provide a calibration curve.

4-Butoxyphenylacetic Acid—Two aqueous solutions, containing 12.5 mcg./ml. (I) and 1.25 mcg./ml. (II) of the acid, were used. From Solution I, 2.0- and 1.0-ml. samples were taken in duplicate; from Solution II, 4.0- and 2.0-ml. samples were taken in duplicate. The subsequent procedure was as already described.

Extraction from Plasma—Various volumes of the standard aqueous bufexamac solutions were added to samples of fresh pig plasma (3 ml.) containing heparin. Tartaric acid (0.5 *M*, 3 ml.) was added to each sample, followed by distilled water to provide a final volume of 9 ml. The subsequent procedure was as described for urine.

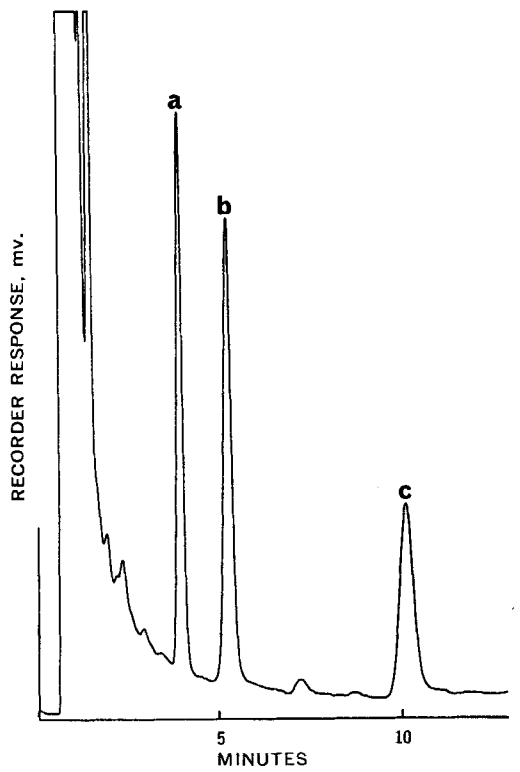


Figure 2—*Gas chromatogram of a mixture of bufexamac, 4-butoxyphenylacetic acid, and 4-butoxyphenylacetamide as their trimethylsilyl derivatives. Conditions were as described in text. Key: a, 4-butoxybenzylisocyanate; b, trimethylsilyl derivative of 4-butoxyphenylacetic acid; and c, trimethylsilyl derivative of 4-butoxyphenylacetamide.*

RESULTS AND DISCUSSION

The relationship between peak height ratio (4-butoxybenzylisocyanate–docosane) and amount of bufexamac was linear within the range studied (2–80 ng. *injected*). This relationship was used to calculate the recovery of bufexamac from urine and plasma, and a similar relationship for 4-butoxyphenylacetic acid was used to calculate the recovery of the acid from urine.

The acetyl, trifluoroacetyl, and trimethylsilyl derivatives of bufexamac, when chromatographed, all gave a single peak, coincident with that obtained from a freshly prepared sample of 4-butoxybenzylisocyanate. This finding suggests that these derivatives, when injected onto the GLC column, undergo the Lossen rearrangement spontaneously, giving 4-butoxybenzylisocyanate. Further evidence to this effect was obtained by interposing a splitter between the end of the column and the detector, so that 80% of the effluent was directed into a trap containing powdered KBr. Repeated injections of a solution of the acetyl derivative of bufexamac in acetone were carried out, and the component with a relative retention of 0.356 was collected. The KBr was pressed into a disk, and the IR spectrum obtained was identical to that of 4-butoxybenzylisocyanate. This appears to be the first example of a trimethylsilyl derivative of a hydroxamic acid undergoing the Lossen reaction. When bufexamac itself is injected, a very small isocyanate peak is obtained.

Vagelos *et al.* (11) reacted hydroxylamine with mixtures of acyl coenzyme A compounds to obtain the corresponding hydroxamic acids. The mixtures of hydroxamates were then acetylated and analyzed as isocyanates by GLC. Several workers observed that the facility of this rearrangement varies according to the nature of the hydroxamic acid derivative (12–15).

In the present study, it was observed that the trimethylsilyl derivative of bufexamac gave twice the response for the isocyanate peak compared with that from the acetyl and trifluoroacetyl derivatives. The trimethylsilyl derivative gave the same response as an equimolar amount of the freshly prepared isocyanate. This indicates that the conversion of this derivative to the isocyanate is virtually quantitative. Variation of the column and injection port temperature between 150 and 250° did not significantly affect the rearrangement. The conditions selected (column and injection heater temperature 220°) were those that gave a retention time (4 min.) suitable for routine analyses. The silylation method has the additional advantage of permitting the derivatization of other substances which might be present in the analytical sample. In this instance, a mixture of bufexamac, 4-butoxyphenylacetic acid, and 4-butoxyphenylacetamide may be estimated separately by the same silylation procedure.

An important advantage of this procedure over current colorimetric methods of analysis for hydroxamates is that any hydroxy metabolite of the drug would probably be silylated and determined

Table II—Recovery of 4-Butoxyphenylacetic Acid from Urine

Micrograms per Milliliter		Mean Percentage Recovery
Added	Found	
16.60	17.70, 17.30	105.4
8.32	8.33, 8.37	100.4
4.16	4.20, 4.29	102.1
1.66	1.46, 1.58	91.7
	Overall recovery	99.9 ± 5.9

as the corresponding isocyanate. The colorimetric methods cannot distinguish between bufexamac and any hydroxy derivatives of bufexamac. No evidence was, in fact, obtained for the presence of a hydroxy derivative of bufexamac as a metabolite of the drug in blood or urine.

The smallest amount of bufexamac that could be assayed following extraction with a standard deviation within the limits reported here corresponded to a concentration of about 0.6 mcg./ml. in urine or plasma. This amount is equivalent to about 2 ng./μl. injected, giving a peak height of about 60 mm. (1×10^{-11} amp. full scale deflection).

An investigation into the general applicability of the Lossen rearrangement to the analysis of hydroxamic acid derivatives of other compounds of pharmaceutical interest is currently being undertaken.

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Reaction of 3-(Chloroalkyl)-2-benzoxazolinones with Amines: Formation of 3-(Aminoalkyl)-2-benzoxazolinones and 5-Substituted-2,3,4,5-tetrahydro-1,5-benzoxazepines

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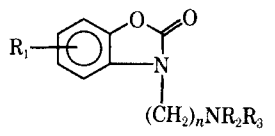
Abstract □ The reactions of 3-(chloroalkyl)-2-benzoxazolinones with various bases are described.

Keyphrases □ 3-(Aminoalkyl)-2-benzoxazolinones—preparation from 3-(chloroalkyl)-2-benzoxazolinones □ 3-(Chloroalkyl)-2-benzoxazolinones—reactions with amines □ 5-Substituted-2,3,4,5-tetrahydro-1,5-benzoxazepines—preparation from 3-(chloroalkyl)-2-benzoxazolinones □ CNS depressants, potential—3-(aminoalkyl)-2-benzoxazolinones, preparation □ IR spectrophotometry—identification, 3-(aminoalkyl)-2-benzoxazolinones □ NMR spectroscopy—identification, 3-(aminoalkyl)-2-benzoxazolinones

The observed CNS depressant activity associated with some 3-(aminoalkyl)-2-benzoxazolinones (I) (1, 2), as well as parenthetic analogy with unbranched aminopropyl side chains in the 10-position of phenothiazines (II) (3), prompted the investigation of other 3-(aminoalkyl)-2-benzoxazolinones.



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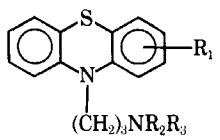
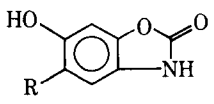


II

Metabolic studies by Bray *et al.* (4) on 2-benzoxazolinone and by Conney and Burns (5) on 5-chloro-2-benzoxazolinone indicated that the major urinary metabolites of both are the 6-hydroxylated compounds



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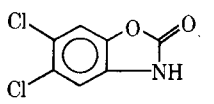

IV

(III). To determine if a hindrance of metabolic detoxication would enhance biological activity of type I compounds, 5,6-dichloro-2-benzoxazolinone (IV) was selected for further study. The introduction of the aminopropyl side chain of the phenothiazines into the 3-position of the parent system was undertaken in anticipation of obtaining interesting CNS depressants.

The preparation of I ($n = 2$ or 3) by the reaction of an appropriately substituted 2-benzoxazolinone with an aminoalkyl halide was described (1, 2, 6). Such synthetic pathways occasionally, however, involve troublesome preparations of the intermediate aminoalkyl halides, thus subjecting the overall synthetic pathways to poor