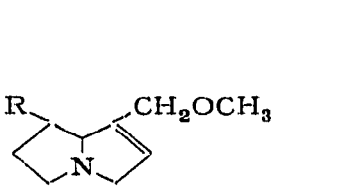
CHARACTERISATION OF PYRROLIZIDINE ALKALOIDS BY GAS, THIN-LAYER AND PAPER CHROMATOGRAPHY

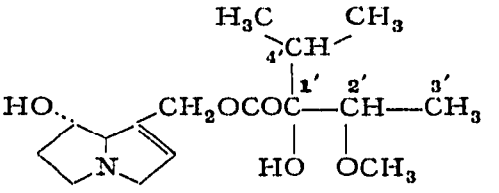
A. H. CHALMERS, C. C. J. CULVENOR AND L. W. SMITH
Division of Organic Chemistry, C.S.I.R.O. Chemical Research Laboratories, Melbourne (Australia)
(Received April 5th, 1965)

From the standpoint of gas chromatography, alkaloids are among the less amenable classes of organic compounds. The technique has been applied to several groups of low molecular weight bases[1-4] including pyrrolizidine derivatives[5,6] but there has been very little usage with bases of high molecular weight following exploratory demonstrations[7,8] that such alkaloids could be chromatographed successfully. The principal difficulty in applying gas chromatography to alkaloids appears to be not lower volatility of these substances, but a higher adsorptivity and an enhanced decomposition rate when they are in contact with certain packing materials and with metal components commonly used in gas-chromatographic apparatus.

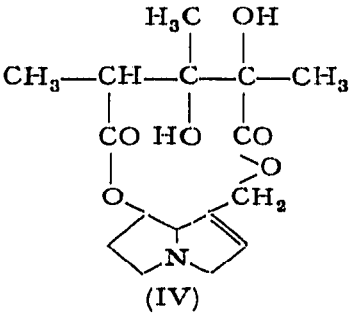
With suitable apparatus we have found that pyrrolizidine alkaloids, including the macrocyclic diesters of melting point above 200°, can be chromatographed readily and with reproducible retention times. The retention times are spread over a very wide range of values and supplement thin-layer and paper chromatography to the point where, for the first time, identity or non-identity of alkaloids in the pyrrolizidine group may be established with a considerable degree of reliability by chromatographic comparison with reference compounds. We report here such characterisation data for 58 alkaloids and basic derivatives.



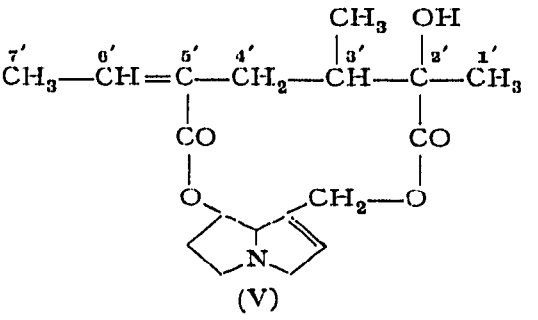
(I) R=H
(II) R=β-OH



(III)



(IV)



(V)

In early experiments with an apparatus composed of a copper column and copper inlet and outlet systems, we found that simple bases such as 1-methoxymethyl-1,2-dehydropyrrolizidine (I) and its 7β-hydroxy derivative (II) could be eluted successfully[5,6], but not ester alkaloids such as heliotrine (III) and monocrotaline (IV). With an apparatus based on that of FALES AND PISANO[2] with all-glass components (apart from the stainless steel micropipette used in introducing samples), even the least volatile ester alkaloids may be eluted with little or no decomposition if attention is given to two aspects. Firstly, the interior of the apparatus, inlet system, column and detector (argon ionisation type), together with the support for the liquid phase must be carefully silanized to eliminate adsorption sites as far as possible. Secondly, it is necessary to apply the alkaloids in solutions of fairly low concentration (approx. 5%, 1–5 μl aliquots). Use of solutions of higher concentration leads to partial decomposition as evidenced by formation of char in the inlet and appearance of comparatively large peaks shortly after the solvent front due to volatile pyrolysis products. Application of gummy or solid alkaloids, as such, enhances decomposition to the point where no peak may be observed at all at the position corresponding to the alkaloid applied.

EXPERIMENTAL

The gas chromatographic apparatus was constructed in the laboratory from glass components, the column being 6 ft. in length and 6 mm I.D. The column packing, 4% SE-30 siloxane polymer on Gas Chrom P was prepared and silanized with dimethyldichlorosilane according to the procedure of FALES AND PISANO[2]. The interior surfaces of the column, inlet and outlet were similarly silanized. The column was operated with inlet pressure 650 mm and at a temperature of 140° for the lower molecular weight, non-ester bases and at 960 mm and 205° for the higher molecular weight bases. Samples were applied as 1–5 μl aliquots of 5 % solution in chloroform or ethanol. The aliquots were introduced by means of a stainless steel capillary and holder which is inserted into a glass throat in the inlet in such a way that the contents of the capillary are immediately and completely expelled by the carrier gas (*cf.* KRANTZ[9]). The capillary is then withdrawn. The inlet is maintained at 250° and includes a short length (*ca.* 2 cm) of silanized Gas Chrom P which collects non-volatile constituents of the sample or carbonaceous pyrolysis products and is replaced at intervals of about one week. An argon ionisation detector was employed.

Thin-layer chromatography was carried out on plates prepared from a slurry of silica gel (Merck "Silicagel G", 30 g) and sodium hydroxide (*N*/10, 60 ml) and kept for at least a day before use. Methanol was the developing solvent and spots were detected in iodine vapour. Paper chromatography was effected on Whatman No. 1 paper, with ascending solvent which was the top layer resulting from shaking *n*-butanol with an equal volume of 5 % acetic acid.

RESULTS

At column temperatures up to 205° (the maximum used), the alkaloids gave peaks which were sufficiently sharp to imply freedom from decomposition during passage through the column itself, even if volatile pyrolysis products appeared im-

mediately after the solvent peak. Thus decomposition is probably restricted to the inlet system (temp., 270°) where it may be catalysed to some degree by the stainless-steel micropipette or by carbonaceous decomposition products of earlier samples. Although all alkaloids have given sharp peaks with newly prepared columns, some are sensitive to a deterioration in conditions. Peaks may then become diffuse with retention time varying with the quantity of alkaloid applied (*e.g.* heliotridine, platynecine, supinidine and isoretronecanol), or the alkaloid peak may be replaced entirely by peaks of lower retention time due to decomposition products (*e.g.* echinatine, retronecine viridiflorate, retronecine trachelanthate and rinderine). Since the former

TABLE I

RELATIVE RETENTION TIMES (AT 140°) AND R_F VALUES OF NON-ESTER PYRROLIZIDINE ALKALOIDS AND DERIVATIVES

For conditions, see EXPERIMENTAL section.

No.	*Base*	*B.P.* (°C/mm)	*M.P.* (°C)	R_T (min)	R_F (T.L.C.)	R_F (paper)
1	1-Methylenepyrrolizidine	115/150	—	1.6	0.05	0.39
2	Heliotridane (1β-methyl-8α-pyrrolizidine)	169/760	—	1.8	0.01	0.39
3	Anhydroplatynecine	194/750	—	2.4	0.05	0.22
4	7β-Hydroxy-1-methylene-8α-pyrrolizidine	41/0.1	35–36	3.3	0.07	0.27
5	Desoxyretronecine (7β-hydroxy-1-methyl-1,2-dehydro-8α-pyrrolizidine)	—	79–80	3.4	0.07	0.31
6	7β-Hydroxy-1-methylene-8β-pyrrolizidine	62/0.03	34–36	4.4	0.14	0.28
7	Retronecanol (7β-hydroxy-1β-methyl-8α-pyrrolizidine)	140/30	98–98.5	4.5	0.01	0.40
8	Hydroxyheliotridane (7α-hydroxy-1β-methyl-8α-pyrrolizidine)	92/0.5	61.5–62.5	4.5	0.02	0.29
9	7α-Hydroxy-1-methyl-1,2-dehydro-8α-pyrrolizidine	114/3.5	67–68	4.5	0.13	0.29
10	1-Methoxymethyl-1,2-dehydro-8α-pyrrolizidine	100/10	—	5.4	0.08	0.36
11	1-Methoxymethyl-1,2-epoxy-pyrrolizidine	53/0.1	—	6.4	0.23	0.32
12	Isoretronecanol (1β-hydroxymethyl-8α-pyrrolizidine)	115–16/1.5	39–40	7.4	0.02	0.24
13	Supinidine (1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine)	90/0.1	29–30	7.4	0.04	0.24
14	1-Hydroxymethyl-1,2-epoxy-8α-pyrrolizidine	80/0.04	—	8.3	0.18	0.20
15	7β-Hydroxy-1-methoxymethyl-1,2-dehydro-8α-pyrrolizidine	77/0.4	36–38	9.0	0.12	0.33
16	7β-Acetoxy-1-methoxymethyl-1,2-dehydro-8α-pyrrolizidine	—	—	15.0	0.30	0.54
17	Retronecine (7β-hydroxy-1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine)	—	117–118	15.0	0.07	0.20
18	Heliotridine (7α-hydroxy-1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine)	—	117–118	15.0	0.14	0.20
19	Platynecine (7β-hydroxy-1β-hydroxymethyl-8α-pyrrolizidine)	—	148–148.5	15.0	0.01	0.21

effect may be partially overcome by raising the column temperature, the effects are probably due to adsorption on, as well as promotion of decomposition by material remaining in the column after passage of earlier samples.

Relative retention times of the alkaloids are shown in Tables I, II and III, along with R_F values measured in the thin-layer and paper chromatographic systems which have been found most satisfactory for general purposes. Non-ester alkaloids and derivatives are run at a column temperature of 140° but the preferred temperature for ester alkaloids is 205°. It is not possible to compare the two series of bases at both temperatures. The esters most readily eluted at 205°, 7-angelylretronecine and 7-angelylheliotridine, are not eluted at all at 140°, while the non-ester bases eluted most slowly at 140°, retronecine and heliotridine, have very short elution times of about 1.5 min at 205°.

Relative retention times for the non-ester bases range from 1.6–15.0 min largely according to molecular weight and number of hydroxyl groups. With the important exception of retronecine and heliotridine, bases with a 7β-OH have lower retention times than 7α-OH isomers.

The two groups of ester alkaloids, the esters of monocarboxylic acids (Table II) and the macrocyclic diesters (Table III) have retention times within similar ranges, 4.2–55.0 min and 13.9–57.0 min respectively. The esters of monocarboxylic acids fall into four discrete subgroups:

(i) the 7-angelyl esters of retronecine and heliotridine, R_T 4.2 and 4.8 min, resp.;

(ii) supinidine esters, heleurine and supinine, R_T 8.0 and 8.8 min resp.;

(iii) monoesters of heliotrine type, R_T 12.4–18.2 min;

(iv) diesters corresponding to 7-angelyloxy esters of group (iii), R_T 29.1–55.0 min.

Within the larger groups (iii) and (iv), the order of retention times appears to depend largely on the number of hydroxyl groups together with the factor already mentioned that alkaloids with a C7-β-OH group have lower retention times than isomers with C7-α-OH.

Grouping of the macrocyclic diesters (Table III) appears to be determined largely by whether the ring is 11- or 12-membered, *i.e.* whether the esterifying acid is a glutaric or adipic acid derivative, and by the number of hydroxyl groups:

(i) 11-membered rings of monocrotaline type, R_T 13.9–19.5 min; excepting spectabiline, R_T [illegible].2 min;

(ii) 12-membered rings of senecionine type with one OH group, R_T 20.6–35.5 min;

(iii) 12-membered rings of senecionine type with two OH groups, R_T 34.6–47.6 min.

The quoted ranges of relative retention times do not include values for the diesters of otonecine which are considerably higher (approx. 50 %, see below) than those of corresponding retronecine diesters. Grantianine also has an exceptionally high retention time, 57.0 min, which appears to be due to the additional lactone function, *cf.* a similarly high R_T for latifoline.

In considering possible correlations between relative retention times and molecular structure of the alkaloids, it was of interest to determine whether the same type of relationship existed as has been established for long chain compounds[10] and for steroids[11,12]. For these classes of compounds, minor structural changes or the introduction of functional groups produce additive effects on the logarithm of the relative retention time. Expressed differently, a particular molecular change has a

TABLE II

RELATIVE RETENTION TIMES (AT 205°) AND R_F VALUES OF PYRROLIZIDINE ESTERS WITH MONOCARBOXYLIC ACIDS

For conditions, see EXPERIMENTAL section.

No.	*Base*	*M.P.* (°C)	R_T (*min*)	R_F (*T.L.C.*)	R_F (*paper*)
20	7-Angelylretronecine	76–77	4.2	0.33	0.49
21	7-Angelylheliotridine	116–117	4.8	0.45	0.52
22	Heleurine	67–68	8.0	0.11	0.50
23	Supinine	148–149	8.8	0.10	0.40
24	Heliotrine	128	12.4	0.30	0.43
25	Indicine	97–98	14.3	0.19	0.38
26	Retronecine trachelanthate	—	14.3	0.19	0.38
27	Retronecine viridiflorate	—	14.3	0.19	0.38
28	Rinderine	100–101	15.6	0.29	0.34
29	Echinatine	109–110	15.6	0.30	0.36
30	Europine	(N-oxide 171)	18.2	0.29	0.34
31	Sarracine	45–46	29.1	0.16	0.56
33	Echiumine	99–100	35.6	0.47	0.65
34	Lasiocarpine	96.5–97	46.4	0.54	0.59
35	Echimidine	(picrate 142–143)	47.6	0.45	0.57
36	Heliosupine	(picrate 103–106)	50.6	0.53	0.51
37	Latifoline	102–103	55.0	0.53	0.54

TABLE III

RELATIVE RETENTION TIMES (AT 205°) AND R_F VALUES FOR MACROCYCLIC DIESTER ALKALOIDS

For conditions, see EXPERIMENTAL section.

No.	*Base*	*M.P.* (°C)	R_T (*min*)	R_F (*T.L.C.*)	R_F (*paper*)
38	Retusine	174–175	13.9	0.16	0.44
39	Fulvine	213.5–214	15.5	0.33	0.43
40	Crispatine	137–138	15.6	0.29	0.39
41	Monocrotaline	202–203	19.5	0.29	0.39
42	Senecionine	245	20.6	0.40	0.56
43	Seneciphylline	217	21.1	0.38	0.50
44	Platyphylline	129	24.0	0.18	0.50
45	Integerrimine	172.5	24.3	0.39	0.57
46	Spectabiline	185.5–186	27.2	0.34	0.43
47	Senkirkine	198	31.6	0.29	0.47
48	Jacobine	228	34.0	0.38	0.36
49	Sceleratine	178	34.6	0.34	0.39
50	Jacozine	228	35.5	0.37	0.32
51	Jacoline	221	36.6	0.37	0.23
52	Rosmarinine	209	37.1	0.35	0.40
53	Jaconine	147	40.4	0.47	0.47
54	Retrorsine	219–220	40.7	0.35	0.36
55	Riddelliine	198	41.9	0.32	0.28
56	Retusamine	174.5	50.3	0.30	0.51
57	Otosenine	221	51.0	0.23	0.34
58	Grantianine	209–209.5	57.0	0.31	0.33

constant proportional effect on relative retention time itself. In Table IV, "structural factors" are derived for the commonest structural modifications which occur in pyrrolizidine alkaloids, such as esterification of the hydroxyl group at C7, change from retronecine to heliotridine configuration, etc. Although the available data allow comparison of only two, three or four examples of any one change, the ratio R_T (modified compound)/R_T (initial compound) is sufficiently constant for most changes to show that this is a valid way of correlating relative retention time with molecular structure, and that the structural factors should have utility in making predictions about new compounds. These conclusions require qualification in respect of the non-ester bases for changes involving the 7-OH groups and for 1-CH_3 → 1-CH_2OH; perhaps because hydrogen bonding has a greater, and less regular, influence in the small molecules, the retention time ratios in these instances are too variable to permit reliable predictions to be made.

Apart from esterification of the C7-OH groups by angelic acid for which a large increase in retention time is to be expected because of the substantial increase in molecular weight, the largest structural factors in both groups of ester alkaloids are those relating to introduction of hydroxyl groups. More surprisingly the factor due to epoxidation of the ethylidene group in macrocyclic alkaloids (1.67) is almost as large as that due to introduction of hydroxyl at C1′ (1.98), and larger than that due to introduction of a tertiary hydroxyl in heliotrine-type esters (1.40). The change from a retronecine to an otonecine nucleus, which has an additional oxygen atom and partial charge separation in the $\overset{\delta+}{N}$–C8–$\overset{\delta-}{O}$ system, also produces a substantial increase in relative retention time (factor 1.52). In general we have refrained from writing structural factors for changes represented by only one example; the exception is the rather important comparison of the *cis* and *trans* ethylidene groupings in macrocyclic diesters. The indication is that the *cis* configuration leads to appreciably lower retention times than the *trans* configuration; the factor given in Table IV (1.18) is supported by comparison of the relative retention time of retrorsine with that of a new alkaloid from *Crotalaria usaramoensis* which, from N.M.R. data (unpublished) is almost certainly the *trans*-isomer of retrorsine (ratio, 1.17).

Thin-layer chromatography on silica gel

The main virtues of thin-layer chromatography as applied to pyrrolizidine alkaloids appear to be speed of characterisation and high sensitivity in detection of impurities or small amounts of the alkaloids. As the two methods were used in the present investigation, thin-layer was more sensitive than gas chromatography, an important factor being that thin-layer plates may be grossly overloaded without marked effect on R_F values if these are measured from the bottom edge of the spot.

In the non-ester group (Table I) R_F values are variable without any general trends being discernible. The esters of monocarboxylic acids, (Table II) have R_F values falling into several groups with remarkably little variation within the groups:

(i) supinidine esters, R_F 0.10–0.11;
(ii) platynecine ester (sarracine), R_F 0.16;
(iii) retronecine monoesters of indicine type, R_F 0.19;
(iv) heliotridine monoesters of heliotrine type, R_F 0.29–0.30;
(v) retronecine diesters, R_F 0.45–0.53;
(vi) heliotridine diesters, R_F 0.53–0.57.

TABLE IV

EFFECTS OF STRUCTURAL CHANGES ON RELATIVE RETENTION TIMES

Structural change	*Examples*[a]	*Ratio* $\frac{R_T \text{ modified compd.}}{R_T \text{ initial compd.}}$	*Structural factor (mean of example ratios)*
Non-ester bases			
1-CH_3 → 1-CH_2OH	2 → 12	4.1	3.8
	5 → 17	4.4	
	7 → 19	3.3	
	9 → 18	3.3	
7-H → 7α-OH	1 → 6[b]	2.75	2.43
	2 → 8	2.50	
	13 → 18	2.03	
7-H → 7β-OH	1 → 4	2.06	2.06
	2 → 7	2.50	
	10 → 15	1.67	
	13 → 17	2.03	
	12 → 19	2.03	
7β-OH → 7α-OH	4 → 6[b]	1.33	1.16
	7 → 8	1.0	
	5 → 9	1.32	
	17 → 18	1.0	
1,2-dehydro → 1,2-epoxy	10 → 11	1.18	1.15
	13 → 14	1.12	
1-CH_2OH → 1-CH_2OMe	13 → 10	0.73	0.70
	14 → 11	0.77	
	17 → 15	0.60	
Esters of monocarboxylic acids[c]			
7α-OH → 7α-Angeloxy	26 → 33	2.49	2.51
	30 → 34	2.54	
7-H → 7α-OH	22 → 24	1.55	1.66
	23 → 28	1.77	
4′-H → 4′-OH	24 → 30	1.47	1.40
	33 → 35	1.34	
7β-Angeloxy → 7α-Angeloxy	20 → 21	1.14	1.10
	26 → 28	1.09	
	27 → 29	1.09	
	35 → 36	1.06	
2′-OH → 2′-OMe	23 → 22	0.91	0.85
	28 → 24	0.80	
Macrocyclic diesters[c]			
1′-H → 1′-OH	42 → 54	1.98	1.98
	43 → 55	1.98	
5′,6′-double bond → 5′,6′-epoxy	42 → 48	1.65	1.67
	43 → 50	1.68	
Retronecine nucleus → otonecine nucleus	42 → 47	1.53	1.52
	48 → 57	1.50	
cis → *trans* 5′,6′-double bond	42 → 45	1.18	1.18
3′(H,CH_3) → 3′(=CH_2)	42 → 43	1.02	1.03
	48 → 50	1.04	
	54 → 55	1.03	

[a] Alkaloid numbers as given in Tables I–III.
[b] Base 6 is the enantiomer of 7α-hydroxy-1-methylene-8α-pyrrolizidine.
[c] Dashed numbers indicate carbon atoms of the esterifying acids; see diagrams (III) and (V).

The macrocyclic diester groups are not as sharply distinguished from each other:

(i) platynecine diester, R_F 0.18;

(ii) otonecine diesters, R_F 0.20–0.29;

(iii) retronecine diesters with an 11-membered ring, R_F 0.29–0.34;

(iv) retronecine diesters with a 12-membered ring, R_F 0.32–0.47.

There is no marked effect of molecular weight or number of hydroxyl groups as in the gas-chromatographic results, the R_F values being determined essentially by the nature of the aminoalcohol and its type of esterification, *i.e.* monoester of monocarboxylic acid, diester of monocarboxylic acids or macrocyclic diester. Since the thin-layer adsorbent is silicic acid it would not be surprising if base strength were an important factor in determining R_F values. The above groupings support this suggestion to some extent in that supinidine and platynecine are more strongly basic (pK_a 10.0, 10.2, respectively[13]) than retronecine and heliotridine (pK_a 8.9, 9.0, respectively[13]) and their esters probably follow the same pattern. There is no other obvious explanation for the low R_F values of supinidine and platynecine esters, which are not similarly distinguished from retronecine and heliotridine esters by paper chromatography in the butanol–acetic acid system.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. H. M. FALES for advice and for first demonstrating the utility of the technique with this series of bases, and to Mr. Z. KRANTZ for constructing and maintaining the apparatus.

SUMMARY

Gas–liquid chromatography of pyrrolizidine alkaloids is described. Retention times are tabulated for 58 alkaloids and derivatives and discussed in relation to the molecular structures of the compounds. Minor structural changes are shown to produce additive effects on the logarithm of the relative retention time as has been found with other classes of compounds. Thin-layer and paper chromatographic data are also recorded.

REFERENCES

1 L. D. QUIN, *Nature*, 182 (1958) 865; *J. Org. Chem.*, 24 (1959) 914.
2 H. M. FALES AND J. J. PISANO, *Anal. Biochem.*, 3 (1962) 337.
3 J. W. FAIRBAIRN, E. J. SHELLARD AND S. TALALAJ, *Planta Med.*, 11 (1963) 92.
4 E. BROCHMANN-HANSSEN AND A. BAERHEIM SVENDSEN, *J. Pharm. Sci.*, 51 (1962) 938.
5 C. C. J. CULVENOR AND L. W. SMITH, *Australian J. Chem.*, 14 (1961) 284.
6 C. C. J. CULVENOR, J. D. MORRISON, A. J. C. NICHOLSON AND L. W. SMITH, *Australian J. Chem.*, 16 (1963) 131.
7 H. A. LLOYD, H. M. FALES, P. F. HIGHET, W. J. A. VANDENHEUVEL AND W. C. WILDMAN, *J. Am. Chem. Soc.*, 82 (1960) 3791.
8 E. BROCHMANN-HANSSEN AND A. BAERHEIM SVENDSEN, *J. Pharm. Sci.*, 51 (1962) 1095.
9 Z. KRANTZ, paper in preparation.
10 F. P. WOODFORD AND C. M. VAN GENT, *J. Lipid Res.*, 1 (1960) 188.
11 W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochim. Biophys. Acta*, 64 (1962) 416.
12 R. J. HAMILTON, W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochim. Biophys. Acta*, 70 (1963) 679.
13 C. C. J. CULVENOR AND R. E. WILLETTE, unpublished data.