dioxane–water (65:35, v/v). A single development took about 6 h at 25°; multiple development, in this particular system, did not result in further resolution. Fig. 1 shows a photograph, taken under U.V. light, of the TLC of the C_1–C_{14} *n*-alkanal DNPH's using the technique described. We have observed that the best separation is achieved with the middle members of the C_1–C_{14} series; the C_1 and C_2 derivatives tend to run together, and the C_{13} and C_{14} derivatives are often not well separated.

Although multiple development did not enhance resolution, we have used what we call "continuous development" to increase resolution. In this technique the derivatives are spotted into the impregnated plates, and the plates are developed with the dioxane–water system with 3–4 cm of the top of the plates exposed to the atmosphere. This is conveniently done in a Saran*-covered 1000 ml beaker, with a slit cut in the Saran film for the plate. Using this technique the slow and medium-mobility fractions are usually well resolved. Overdevelopment, however, can cause the "piling-up" of the fast-moving fractions at the top of the plate. Fig. 2 shows a plate that had been run with "continuous development".

Department of Food Science and Technology,
Oregon State University,
Corvallis, Oreg. (U.S.A.)

L. M. LIBBEY
E. A. DAY

[1] G. URBACH, *J. Chromatog.*, 12 (1963) 196.
[2] F. KLEIN AND K. DE JONG, *Rec. Trav. Chim.*, 75 (1956) 1285.

Received September 12th, 1963

* Saran is a trade name for polyvinylidene chloride.

Thin-layer chromatography of tetra- and pentacyclic triterpenes

Thin-layer chromatography has occasionally been applied to the separation of triterpenes, *e.g.* by TSCHESCHE[1,2] to those of *Bredemeyera floribunda*, by THOMAS[3] to those of *Commiphora glandulosa* and by HUNECK[4] to those of *Sorbus torminalis*.

In experiments with Israeli peat, which will be reported elsewhere, we have developed a system that proved useful in the separation of triterpenoid compounds and permitted their easy identification. The solvent mixture used was heptane–benzene–ethanol (50:50:0.5), applied to alumina G. This mixture has the advantage that an increase in the alcohol concentration increases and a decrease in its concentration decreases the rate of migration. For example, the R_F values for betulin (No. 3) are 0, 0.14, 0.73 for 0 %, 0.5 % and 2 % alcohol, for lupeol (No. 10) 0.16, 0.37, 0.94 for the same three alcohol concentrations.

A systematic study has given the following results, which will be extended by further investigations: *epi*-β-Amyrin (No. 15) and *epi*-lupeol (14) can be separated from their diastereoisomers β-amyrin (No. 6) and lupeol (10); the *epi*-compounds have higher R_F values.

Friedelin (19) can be easily separated from friedelan-3β-ol (13), euphone (31) from euphol (24), allobetulone (17) from allobetulin (11). In these cases, the ketones have higher R_F values than the corresponding secondary alcohols. Equally, the esters of alcohols have higher R_F values than the free alcohols.

In Tables I and II are listed the tetra- and pentacyclic triterpenes so far studied, together with their R_F values and the colours obtained by spraying with three reagents. For convenience, the structural formulae of the compounds investigated are given in Fig. 1. In the tables, the compounds are arranged in the order of increasing R_F.

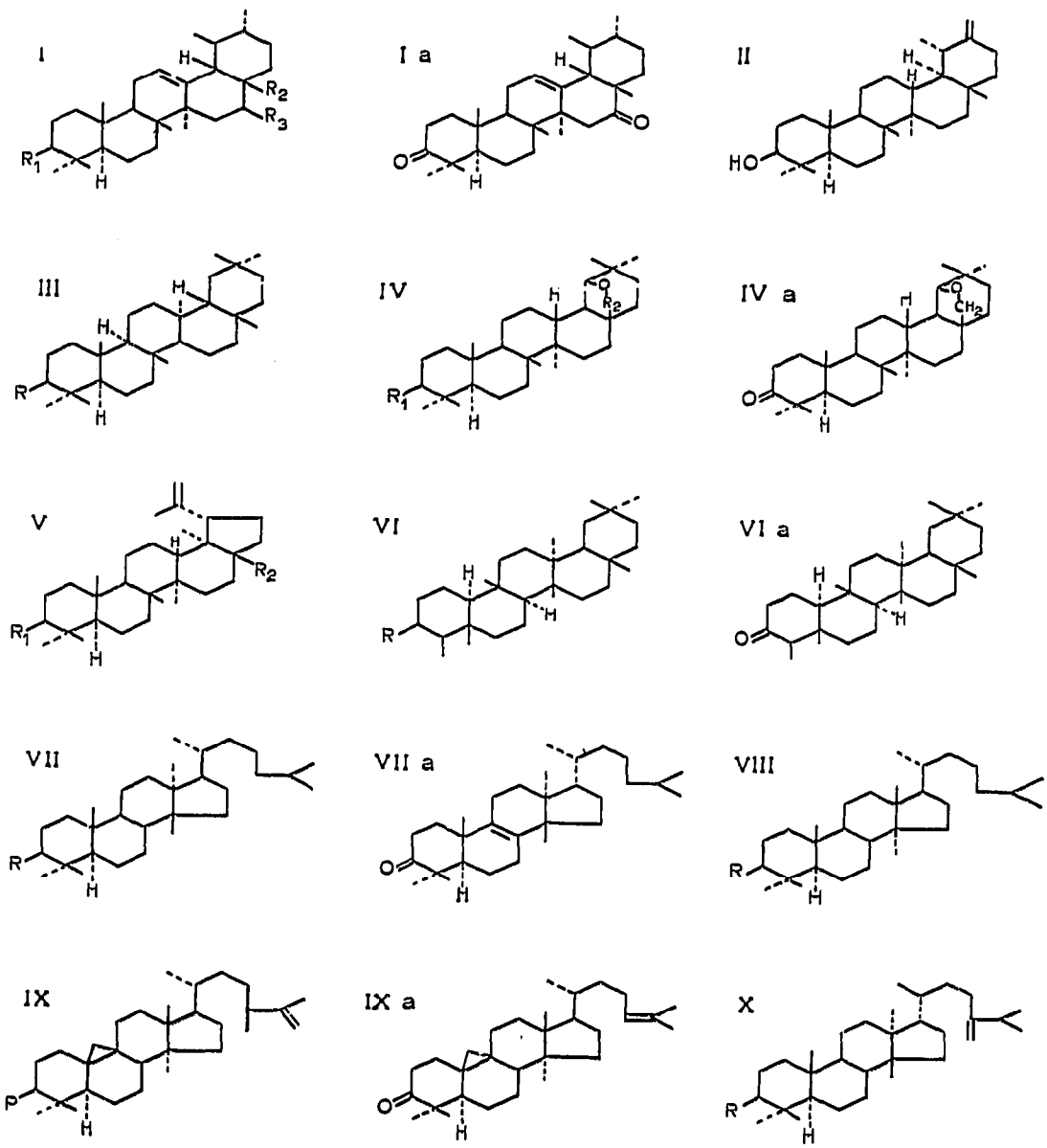

Fig. 1.

In the α-amyrin (I) series, the R_F value decreases with the increasing number of hydroxyl groups. This is also evident in other series, *e.g.* (V). For the β-amyrin (III) compounds, the double bond has a small, but significant effect. If R = OH, the R_F

TABLE I

PENTACYCLIC TRITERPENES

No.	*Compound*	*Structure*	*Substituents*	R_F	$SbCl_3$	$SbCl_5$	Ac_2O/H_2SO_4
1	Uvaol	I	$R_1 = OH$, $R_2 = CH_2OH$, $R_3 = H$	0.03	Blue	Violet	Violet
2	Brein	I	$R_1 = OH$, $R_2 = CH_3$, $R_3 = OH$	0.04	Blue-yellow	Gray	Blue
3	Betulin	V	$R_1 = OH$, $R_2 = CH_2OH$	0.14	Violet	Pink	Violet
4	Taraxasterol	II		0.15	Violet-gray	Violet	Pink
5	Germanicol	III	R = OH, Δ18,19	0.17	Violet	Violet	Pink
6	β-Amyrin	III	R = OH, Δ12,13	0.24	Brown-gray	Pink	Pink
7	α-Amyrin	I	$R_1 = OH$, $R_2 = CH_3$, $R_3 = H$	0.26	Brown-orange	Pink	Pink
8	Taraxerol	III	R = OH, Δ14,15	0.30	Gray	Violet	Pink
9	Dihydrotaraxerol	III	R = OH	0.35	Gray-violet	Brown	Pale brown
10	Lupeol	V	$R_1 = OH$, $R_2 = CH_3$	0.37	Violet-orange	Violet	Violet
11	Allobetulin	IV	$R_1 = OH$, $R_2 = CH_2$	0.38	Yellow	Brown	Yellow
12	Breindione	Ia		0.40	Brown	Yellow	Pink
13	Friedelan-3β-ol	VI	R = OH	0.50	Violet	Violet	Pale brown
14	*epi*-Lupeol	V	$R_1 = \alpha OH$, $R_2 = CH_3$	0.52	Brown	Violet	Brown
15	*epi*-β-Amyrin	III	R = αOH, Δ12,13	0.53	Brown-gray	Violet	Pale brown
16	Oxyallobetulin	IV	$R_1 = OH$, $R_2 = (C = O)$	0.55	Pale brown	Brown	Yellow
17	Allobetulone	IVa		0.78	Pale brown	Brown	Yellow brown
18	β-Amyrin acetate	III	R = OAc, Δ12,13	0.87	Brown	Violet	Violet
19	Friedelin	VIa		0.88	Brown	Brown	Pale brown
20	*epi*-Friedelanyl acetate	VI	R = OAc	0.89	Violet	Brown	Pale brown
21	Taraxeryl acetate	III	R = OAc, Δ14,15	0.91	Gray	Violet	Violet
22	Allobetulin acetate	IV	$R_1 = OAc$, $R_2 = CH_2$	0.91	Violet	Brown	Pale brown
23	β-Amyrin benzoate	III	R = OBz, Δ12,13	0.92	Brown-orange	Violet	Brown

TABLE II

TETRACYCLIC TRITERPENES

No.	*Compound*	*Structure*	*Substituents*	R_F	$SbCl_3$	$SbCl_5$	Ac_2O/H_2SO_4
24	Euphol	VII	R = OH, Δ8,9, Δ24,25	0.20	Brown	Brown	Violet-gray
25	Parkeol	VII	R = OH, Δ9,11, Δ24,25	0.20	Brown	Brown	Violet-gray
26	Cyclolaudenol	IX	R = OH	0.21	Gray	Brown	Violet-gray
27	Butyrospermol	VII	R = OH, Δ7,8, Δ24,25	0.22	Yellow	Violet	—
28	α-Euphorbol	X	R = OH, Δ8,9	0.24	Brown-gray	Red	Violet
29	Lanosterol	VIII	R = OH, Δ8,9, Δ24,25	0.33	Yellow	Brown	Violet
30	Cycloartenone	IXa		0.67	Yellow	Brown	Brown
31	Euphone	VIIa		0.73	Yellow	Yellow-brown	Brown
32	Butyrospermone	VII	R = C=O, Δ7,8, Δ24,25	0.78	Yellow	Brown	Gray
33	Agnosterol	VIII	R = OH, Δ6,7, Δ9,11, Δ24,25	0.88	Yellow	Yellow	Brown-yellow
34	Dihydrobutyrospermyl acetate	VII	R = OAc, Δ7,8	0.90	Gray	Red	Violet
35	Euphene	VIIa	$=CH_2$ instead of C=O	0.96	Brown	Gray-brown	Brown-yellow

value increases when the double bond is transposed from the 18, 19 (No. 5) *via* the 12, 13 (No. 6) to the 14, 15 position (No. 8). The R_F value for the saturated analogue (No. 9) is still higher. This appears to indicate that in the three unsaturated substances the double bonds become less polar or less important for adsorption in the sequence given.

In the tetracyclic series, (VII) has almost the same R_F value, whether the double bonds are in the 24, 25 and the 8, 9 positions (No. 24), in the 24, 25 and the 7, 8 positions (No. 27) or in the 24, 25 and the 9, 11 positions (No. 25).

It is somewhat surprising on the other hand that in the lanosterol series (VIII), three double bonds (No. 33) make the compound migrate more quickly than two (No. 25, 26).

Undoubtedly, a more complete study of this class of compounds will reveal the inherent regularities more clearly.

Experimental procedure

For the preparation of 5 glass plates (20 × 20 cm) a mixture of 50 g of alumina G (Merck) and 100 ml of distilled water was used. The well-shaken mixture was applied to a thickness of 0.25 mm with a Desaga apparatus. After 1 h at room temperature, the plates were dried for 30 min at 125° and kept in a desiccator.

The base line was fixed at a distance of 3 cm from the rim of the plate and the compounds were applied in chloroform solution by means of a micro-pipette. The distance between samples on the same plate was about 2 cm.

The development of the chromatogram with the above-mentioned mixture was carried out in one dimension, at 23°. Within 1 h, the solvent rose a distance of 12 cm. At the end of the development, the height to which the liquid rose was noted; after a further 10 min at room temperature, the plates were dried for 5 min at 120°.

The triterpenes were detected by spraying with three reagents: (A) antimony trichloride, 20% in chloroform, (B) antimony pentachloride, 20% in chloroform, (C) acetic anhydride (10%) and sulphuric acid (10%) in absolute alcohol.

After spraying, the plates were dried at 120° for 5 min.

Acknowledgements

The authors wish to thank Drs. J. McLEAN and W. LAWRIE of the Royal College of Science and Technology, Glasgow, for samples of the triterpenes.

Department of Organic Chemistry, Hebrew University, Jerusalem (Israel)

R. IKAN
J. KASHMAN
E. D. BERGMANN

[1] R. TSCHESCHE, F. LAMBERT AND G. SNATZKE, *J. Chromatog.*, 5 (1961) 217.
[2] R. TSCHESCHE AND A. K. SEN GUPTA, *Ber.*, 93 (1960) 1903.
[3] A. F. THOMAS AND I. M. MULLER, *Experientia*, 16 (1960) 62.
[4] S. HUNECK, *J. Chromatog.*, 7 (1962) 561.

Received August 27th, 1963