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**Note**

# Gas–liquid chromatographic separation of oxidation products of triterpene pentacyclic alcohols

BOGUSŁAW WIŁKOMIRSKI and ZOFIA KASPRZYK
*Institute of Biochemistry, University of Warsaw, Żwirki i Wigury 93, 02-089 Warsaw (Poland)*
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Studies of the triterpene pentacyclic alcohols from *Calendula officinalis* flowers have demonstrated the presence of mono- and di-hydroxy compounds with ursane, oleanane and lupane skeletons[1], and we have shown[2] that gas–liquid chromatography (GLC) of the mixture of free monools and their acetates allows the separation and quantitative determination of $\beta$-amyrin, taraxasterol, $\psi$-taraxasterol and the sum of $\alpha$-amyrin and lupeol.

The present study describes the conditions permitting the GLC separation of $\alpha$-amyrin from lupeol after oxidation of the latter with selenium dioxide; the separation of triterpene diols, their acetates and their oxidation products by GLC is also described.

## EXPERIMENTAL

### *Materials*

Triterpene monools and diols obtained from *Calendula officinalis* flowers were acetylated and then oxidized by reaction with selenium dioxide.

### *Acetylation*

Triterpene alcohol acetates were prepared as before[2].

### *Oxidation with selenium dioxide*

The triterpene alcohol acetate (1 mg) was dissolved in 0.3 ml of anhydrous benzene, 10 mg of freshly sublimed selenium dioxide and 0.6 ml of anhydrous acetic acid were added, and the mixture was heated at 100° for periods varying from 60 to 150 min; the mixture was then diluted to 5 ml with water and extracted with diethyl ether.

### *Gas–liquid chromatography*

The GLC was carried out on a Pye-Unicam 104 instrument equipped with a flame ionization detector and a 5 ft. × 4 mm I.D. glass column of 1.5% of OV-17 on Shimalite W (Shimadzu Seisakusho, Kyoto, Japan); the operating conditions were as follows: column temperature 294°, detector temperature 300°, carrier gas (nitrogen) flow-rate 30 ml/min.

TABLE I

RELATIVE RETENTION TIMES OF SELENIUM DIOXIDE OXIDATION PRODUCTS OF *CALENDULA OFFICINALIS* MONOOL ACETATES

| *Oxidation product of* | *OV-17 column (294°)* * |
|---|---|
| (1) β-Amyrin | 1.96 |
| (2) α-Amyrin** | 2.15 |
| (3) Lupeol | 4.30 |
| (4) ψ-Taraxasterol | 6.21 |
| (5) Taraxasterol | 6.21 |
| (6) Mixture of 1–5 | |
| Peak 1 | 1.93 |
| Peak 2 | 2.14 |
| Peak 3 | 4.21 |
| Peak 4 | 6.21 |

* Internal standard: cholesterol.
** Not oxidised.

## RESULTS AND DISCUSSION

Among the triterpene pentacyclic monools from *Calendula officinalis* flowers, only α-amyrin is not oxidized by the reaction with selenium dioxide[3]. Oxidation of β-amyrin acetate with selenium dioxide yielded olean-11,13(18)-dien-3β-ol acetate ($\lambda_{max.}$ in ethanol, 242, 250 and 260 nm). Oxidation of lupeol acetate yielded 30-oxolupa-20(29)-en-3β-ol acetate ($\lambda_{max.}$ in ethanol, 225 nm), and oxidation of ψ-taraxasterol or taraxasterol yielded, as expected, 30-oxoursa-20-en-3β-ol acetate($\lambda_{max.}$ in ethanol, 232 nm).

The oxidation products of monools were separated on the OV-17 column; their retention times relative to cholesterol are given in Table I. A mixture of monool oxidation products separated into four peaks corresponding to derivatives of β-amyrin, lupeol, taraxasterol with ψ-taraxasterol and unoxidized α-amyrin.

The yields of the oxidation products of the individual monools are shown in Table II, from which it can be seen that the best yield of the oxidation product of β-amyrin was obtained after reaction for 60 min; the corresponding time for lupeol was 90 min, and that for taraxasterol or ψ-taraxasterol was 120 min. However, during these times, the yields of products differed greatly, being high (83–90%) for β-amyrin, taraxasterol and ψ-taraxasterol and low (33%) for lupeol.

Free diols and their acetates were also separated on the OV-17 column; the

TABLE II

YIELD OF SELENIUM DIOXIDE OXIDATION PRODUCTS FROM MONOOLS AS FUNCTION OF TIME

| *Oxidation product of* | *Yield (%) of product after reaction for* | | | | | |
|---|---|---|---|---|---|---|
| | *60 min* | *75 min* | *90 min* | *105 min* | *120 min* | *135 min* |
| β-Amyrin | 83 | — | 67 | — | — | — |
| Lupeol | 25 | — | 33 | — | 15 | — |
| Taraxasterol or ψ-taraxasterol | — | — | — | 87 | 90 | 71 |

TABLE III

RELATIVE RETENTION TIMES OF FREE DIOLS AND THEIR ACETATES ON OV-17

| *Derivative of* | *Free** | *Acetate** |
|---|---|---|
| (1) Erythrodiol | 3.00 | 3.39 |
| (2) Brein | 3.44 | 3.53 |
| (3) Ursadiol | 3.44 | 3.78 |
| (4) Calenduladiol | 4.05 | 4.40 |
| (5) Faradiol | 5.09 | 5.66 |
| (6) Mixture of 1–5 | | |
| Peak 1 | 3.03 | 3.53 |
| Peak 2 | 3.44 | 3.88 |
| Peak 3 | 4.05 | 4.59 |
| Peak 4 | 5.09 | 5.65 |

* Internal standard: cholesterol.

results are shown in Table III, from which it can be seen that a mixture of the free diols was separated into four peaks corresponding to (1) erythrodiol, (2) brein and ursadiol, (3) calenduladiol, and (4) faradiol. A mixture of the diol diacetates was also resolved into four peaks, *i.e.*, (1) brein and erythrodiol, (2) ursadiol, (3) calenduladiol, and (4) faradiol.

Of the triterpene pentacyclic diols, brein and ursadiol are not oxidized by selenium dioxide. Faradiol diacetate oxidized with selenium dioxide yielded 30-oxoursa-12-en-3$\beta$,16$\beta$-diol diacetate ($\lambda_{max.}$ in ethanol, 233 nm). Erythrodiol diacetate after oxidation yielded olean-11,13(18)-dien-3$\beta$,28$\beta$-diol diacetate ($\lambda_{max.}$ in ethanol, 242, 251 and 260 nm). Oxidation of calenduladiol diacetate yielded 30-oxolupa-20'-(29)-en-3$\beta$,12$\beta$-diol diacetate ($\lambda_{max.}$ in ethanol, 224 nm). The results of the separation on the OV-17 column of the oxidation products of pentacyclic diols are presented in Table IV. The mixture of diol oxidation products containing the unoxidizable brein and ursadiol was resolved into four peaks corresponding to derivatives of (1) erythrodiol and brein, (2) ursadiol, (3) calenduladiol, and (4) faradiol.

The yields of the oxidation reaction are shown in Table V; the best yields from

TABLE IV

RELATIVE RETENTION TIMES OF DIOL DIACETATES OXIDATION PRODUCTS

| *Oxidation product of* | *OV-17 column (294°)** |
|---|---|
| (1) Erythrodiol | 3.41 |
| (2) Brein** | 3.44 |
| (3) Ursadiol** | 3.80 |
| (4) Calenduladiol | 8.44 |
| (5) Faradiol | 11.00 |
| (6) Mixture of 1–5 | |
| Peak 1 | 3.41 |
| Peak 2 | 3.80 |
| Peak 3 | 8.44 |
| Peak 4 | 11.00 |

* Internal standard: cholesterol.
** Not oxidized.

TABLE V

YIELD OF OXIDATION PRODUCTS FROM DIOL DIACETATES AS FUNCTION OF TIME

| *Oxidation product of* | *Yield (%) of product after reaction for* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *60 min* | *75 min* | *90 min* | *105 min* | *120 min* | *135 min* | *150 min* |
| Erythrodiol | — | 100 | 59 | — | — | — | — |
| Calenduladiol | 27 | — | 23 | — | 8 | — | — |
| Faradiol | — | — | — | — | 67 | — | 85 |

erythrodiol, calenduladiol and faradiol were obtained after 75, 60 and 150 min, respectively.

As with the triterpene monools, the oxidation products of the compounds with ursane and oleanane skeletons were obtained in good yield (85–100%), while the yield of oxidation products from compounds with a lupane skeleton (both monool and diol) was much lower (about 30%).

## CONCLUSION

Analysis of pentacyclic monools and diols or their acetates, and analysis of their oxidation products, permits quantitative determination of each triterpene monool and diol in the fraction isolated from *Calendula officinalis* flowers.

## REFERENCES

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