Journal of Chromatography, 194 (1980) 239–244
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,722

Note

Intermediate- and large-scale reversed-phase preparative high-performance liquid chromatography on an axially compressed column: a facile, quantitative separation of 7α- and 7β-methyl-17β-acetoxy-3-oxoandrost-4-enes*

F. GASPARRINI*, S. CACCHI, L. CAGLIOTI, D. MISITI and M. GIOVANNOLI
Istituto di Chimica Organica, Via Castro Laurenziano 9, 00161 Rome (Italy)
(Received February 4th, 1980)

Analytical high-performance liquid chromatography (HPLC) is a widely applied technique[1–3] and its obvious extension, preparative HPLC, is now well developed and a number of efficient commercial units are available. The high performance of this technique may be utilized successfully in the quantitative separation of complex reaction mixtures, purification of compounds with low α values being readily achieved. Some papers concerning the application of normal-phase preparative HPLC to a number of difficult separations, using radial compressed cartridges (5 × 30 cm), have been published[4–7].

Some separations on pre-packed commercially available semi-preparative columns using both normal and reversed phases have also been reported[8–12]. However, it must be emphasized that all of the above separation procedures use pre-packed commercially available cartridges or columns of fixed length and supports and therefore they are less versatile than a system which allows one to pack columns of convenient lengths with suitable supports.

Such a separation system, using axial compression[13,14], has recently been introduced with 2- and 4-cm I.D. columns by Jobin-Yvon.

Here we report the application of reversed-phase preparative HPLC on axially compressed columns to the separation of 7α- and 7β-methyl derivatives of 17β-acetoxy-3-oxoandrost-4-ene and side-reaction products obtained by reaction of 17β-acetoxy-3-oxoandrosta-4,6-diene with lithium dimethylcuprate.

EXPERIMENTAL

Solvents for analytical separations (methanol, ethyl acetate and *n*-hexane) were of LiChrosolv grade (Merck, Darmstadt, G.F.R.), and the water used was doubly distilled and deionized. Methanol for preparative purposes was of RPE-ACS grade (Carlo Erba, Milan, Italy).

Analytical separations were performed on columns pre-packed (Policonsult, Rome, Italy) with LiChrosorb Si-60 (10 μm), LiChrosorb RP-8 (10 μm) and LiChrosorb RP-18 (7 μm) (Merck) using a Waters Model ALC/GPC-202 chromatograph (Waters

* Dedicated to Professor L. Panizzi on the occasion of his 70th birthday.

0021-9673/80/0000–0000/$02.25

Assoc., Milford, MA, U.S.A.) equipped with a U6-K universal injector, a Model M6000 solvent delivery system, a Model 450 differential UV detector and a Model 401 refractive index (RI) detector.

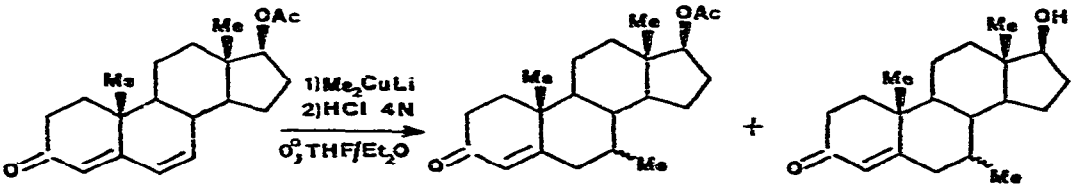
Preparative separations were performed on columns packed with LiChrosorb RP-18 (10 μm) and LiChroprep RP-18 (25–40 μm) (Merck) using a Miniprep LC (2-cm I.D. column) or a Chromatospac Prep 10 chromatograph (4-cm I.D. column), both from Jobin-Yvon (Longjumeau, France), equipped with an RI detector.

The preparative column was packed as follows. A suspension of the desired amount of packing material [LiChrosorb RP-18 (10 μm), LiChroprep RP-18 (25–40 μm)] in methanol–0.1% sodium acetate solution (80:20) was maintained in a ultrasonic bath for 5 min, poured into the column, axially compressed until a selected packing pressure was reached, and finally conditioned by passing 2–3 times the interstitial volume of eluent. The same column can be used successfully for several injections and can be easily regenerated by passing 2–3 times the interstitial volume of methanol or acetonitrile. In order to recover the packing material, the injector was removed from the column and the piston was allowed to push out the compressed adsorbent.

Generally, the loading capacities for 2- and 4-cm I.D. columns lie in the ranges 1 mg–1 g and 50 mg–10 g, respectively.

RESULTS AND DISCUSSION

17β-Acetoxy-3-oxoandrosta-4,6-diene (1)[15] reacts with lithium dimethylcuprate (Me_2CuLi) to give 7α- and 7β-epimeric compounds (2) in 77% overall yield* (53:47 epimeric ratio) through regioselective 1,6-conjugate addition[16,17]. The main isolated by-products (15% overall yield)* are the two epimeric compounds (3), presumably derived from hydrolytic decomposition of (2) during the work-up (Scheme 1).



1 2: α(41%) + β(35%) 3: α(7%) + β(8%)

Scheme 1

In a typical small-scale run, to a stirred suspension of purified copper(I) iodide (2.32 g, 12.15 mmol) in anhydrous diethyl ether (13 ml), 1.64 *M* methyllithium (15 ml) in diethyl ether was added at a temperature not exceeding 0 °C and under argon. Then, at the same temperature, was added a tetrahydrofuran solution (5 ml) of 17β-acetoxy-3-oxoandrosta-4,6-diene (0.5 g, 1.52 mmol). After 10 min the reaction mixture was syphoned into stirred 4 *N* hydrochloric acid and extracted with diethyl ether.

* Yields were calculated using the external standard method.

The organic layer was separated, treated with ammonia–ammonium chloride buffer (pH 8), washed with water and dried over anhydrous sodium sulphate. The solvent was removed at reduced pressure to leave a solid residue (0.490 g), which was analysed by analytical normal- and reversed-phase HPLC under isocratic conditions (Figs. 1 and 2). The best separation of the components of the reaction mixture was obtained by using reversed-phase conditions mainly with an RP-18 support, as shown in Fig. 2a. Even using an RP-8 column a good separation was obtained (Fig. 2b); however, the utilization of an RP-18 column allows a shorter analysis time and a higher selectivity. As a further advantage, the separation on an RP-18 column utilized a mobile phase enriched in methanol; from a preparative standpoint this fact increases the loading of the column.

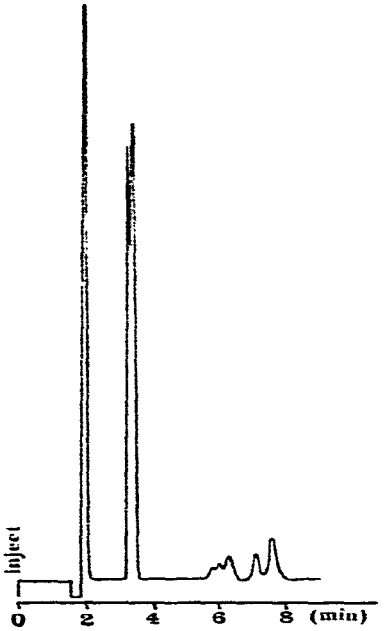


Fig. 1. Results for reaction mixture using normal-phase analytical LC conditions. Packing: LiChrosorb Si-60 (7 μm). Column: 25 cm × 3.0 mm. Solvent: *n*-hexane–ethyl acetate (85:15). Flow-rate: 1.0 ml/min. Detector: RI (×32) Temperature: ambient.

Even the preparative separation was effected by reversed-phase HPLC using axially compressed, high-efficiency 2- or 4-cm I.D. columns, packed with LiChrosorb RP-18 (10 μm) or LiChroprep RP-18 (25–40 μm), respectively. The separation attained with the 2-cm I.D. column is illustrated in Fig. 3a, while the corresponding analytical separation, using the same eluent, is shown in Fig. 3b.

It must be emphasized that the preparative chromatographic conditions were selected in order to achieve a good separation of the main components with the lowest waste of solvent and time (about 0.7 g of mixture were purified in less than 50 min on the 4-cm I.D. column). Under the reported conditions a 95% recovery of products with purity greater than 99% was attained.

The isolated compounds (2α) and (2β) were identified from elemental analysis

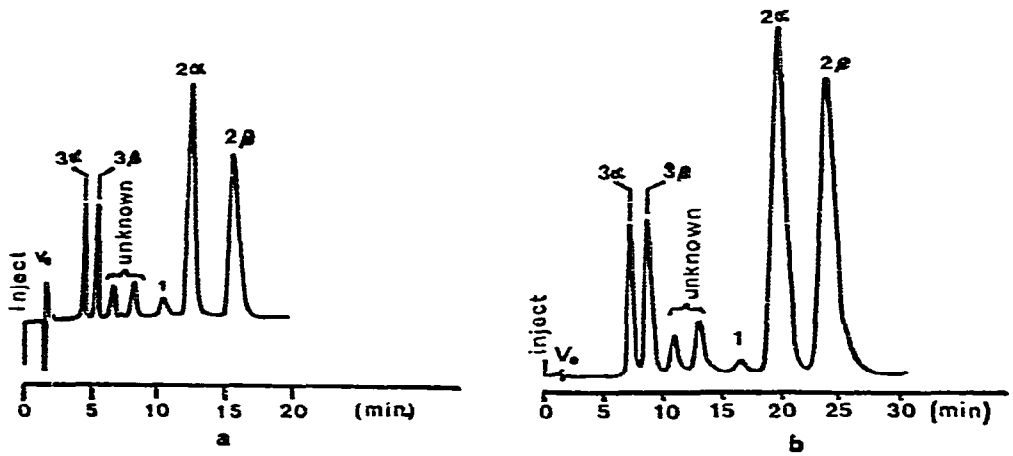


Fig. 2. Results for reaction mixture using reversed-phase analytical LC conditions. (a) Packing: LiChrosorb RP-18 (7 μm). Column: 25 cm × 4.6 mm I.D. Solvent: methanol–water (75:25). Flow-rate: 2.0 ml/min. Detector: RI (×8). Temperature: ambient. (b) Packing: LiChrosorb RP-8 (10 μm). Column: 25 cm × 4.6 mm I.D. Solvent: methanol–water (70:30). Flow-rate: 2.0 ml/min. Detector: RI (×8). Temperature: ambient.

and spectral data. The configuration of the 7-methyl groups was determined by comparison of ^1H and ^{13}C NMR spectra of the isolated products with those of known products of similar structure, such as 17β-hydroxy-7β,17-dimethyl-3-oxoandrost-4-ene (calusterone) (4β)[18] and 17β-hydroxy-7α,17-dimethyl-3-oxoandrost-4-ene (bolasterone) (4α)[18]. The data are listed in Table I.

The structures of the by-products (3α) and (3β) were determined from their mass and ^1H NMR spectra and by comparison with products derived from hydrol-

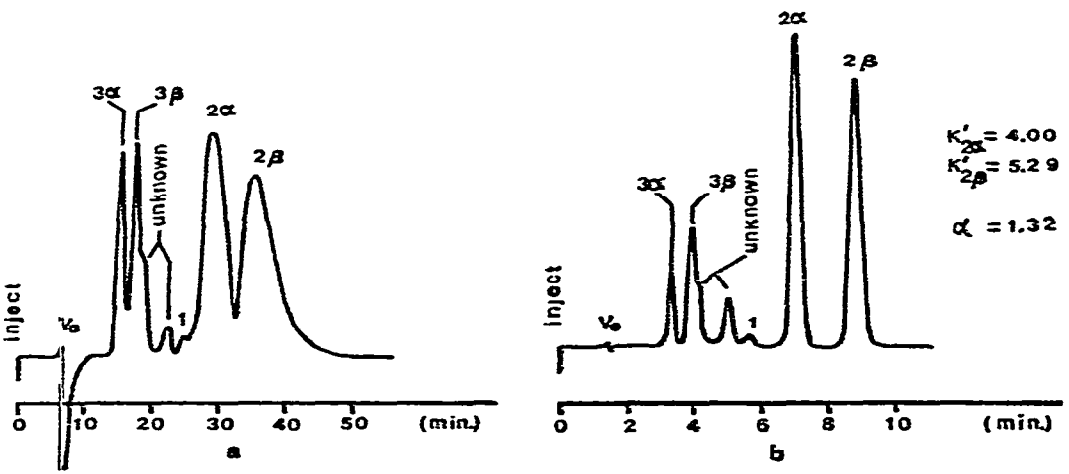


Fig. 3. (a) Result for reaction mixture using preparative LC conditions. Packing: LiChrosorb RP-18 (10 μm), 30 g. Column: 20 cm × 2.0 cm I.D. (P_i = 13 bar). Solvent: methanol–water (80:20). Detector: RI (×20). Flow-rate: 5.5 ml/min (P_e = 7.2 bar). Amount: 0.120 g (1.2 ml of methanol). Temperature: ambient. (b) Results for reaction mixture using analytical LC conditions. Packing: LiChrosorb RP-18 (10 μm). Column: 25 cm × 4.6 mm I.D. Solvent: methanol–water (80:20). Detector: RI (×8). Flow-rate: 2.0 ml/min. Temperature: ambient.

TABLE I

SIENIFICANT ^1H AND ^{13}C NMR CHEMICAL SHIFTS[19] OF THE MAIN PRODUCTS ISOLATED THROUGH PREPARATIVE HPLC

d = doublet; bs = broad singlet.

Compound No.	*^1H NMR ($CDCl_3$) (ppm, TMS)*		*^{13}C NMR ($CDCl_3$) (ppm, TMS)*			*α_D**	*m.p.** (°C)*
	H-4	*7-CH_3*	*7-CH_3*	*CH_3-18*	*CH_3-19*		
4α	5.73 (d, J = 1.8 Hz)	0.75 (d, J = 7 Hz)	12.6	13.8	17.8	+88[18]	158–160[18]
2α	5.72 (d, J = 1.8 Hz)	0.81 (d, J = 7 Hz)	11.9 or 12.7	12.7 or 11.9	17.8	+89	Oil
4β	5.70 (bs, $W_{\frac{1}{2}}$ = 3 Hz)	1.03 (d, J = 5 Hz)	16.5	13.2	21.9	+56[18]	125–127[18]
2β	5.70 (bs, $W_{\frac{1}{2}}$ = 3 Hz)	1.03 (d, J = 5 Hz)	17.5	12.2	22.9	+79	142–143

* Rotations were determined in chloroform in 1-dm tubes at concentrations of 0.8–1.2 mg/ml.

** Melting points are uncorrected and were determined with a Büchi apparatus.

ysis of (2α) and (2β). The unreacted starting material was identified by its capacity factor (k') and the UV spectrum of the eluted peak in analytical HPLC (see Fig. 2a).

Analogous results were obtained in the purification of mixtures derived from 1,6-conjugate addition to 17β-hydroxy-17-methyl-3-oxoandrosta-4,6-diene[18,20] and 11β,17β-dihydroxy-17-methyl-3-oxoandrosta-4,6-diene[18].

CONCLUSIONS

The simple and fast separation procedure described may be proposed as useful substitute for pre-packed systems when complex reaction mixtures must be purified. The high efficiency of axially compressed columns allows a peak resolution comparable to that obtainable under analytical conditions; thus, the isolation of reaction products of high purity and in almost quantitative yield may be easily attained. Even by-products and trace amounts of impurities can be isolated. Further, as the utilization of 4- or 8-cm I.D. columns makes "large-scale" separations feasible, this method may be the separation procedure of choice for the purification of expensive fine chemicals.

Work is in progress to extend the application of axially compressed columns to the purication of complex mixtures of organometallic compounds, carbohydrates, antibiotics, etc., using a number of functionalized bonded phases (diol, CN, NH_2, etc.)[21].

ACKNOWLEDGEMENTS

We thank Dr. Giovanna Cancelliere and Dr. Antonio Arcadi for their technical assistance.

REFERENCES

1 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974.

2 S. G. Perry, R. Amos and P. I. Brewer, *Practical Liquid Chromatography*, Plenum/Rosetta, New York, 1973.

3 G. L. Hawk, *Biological/Biomedical Applications of Liquid Chromatography*, Marcel Dekker, New York, 1979.
4 M. J. Pettei, F. G. Pilkiewicz, K. Nakanishi, *Tetrahedron Lett.*, (1977) 2083.
5 M. A. Adams and K. Nakanishi, *J. Liquid Chromatogr.*, 2 (1979) 1097.
6 S. S. Singer and G. M. Singer, *J. Liquid Chromatogr.*, 2 (1979) 1219.
7 W. M. Waddel, M. M. Dawson, D. L. Hopkins, K. L. Rach, M. Vemura and J. L. Wost, *J. Liquid Chromatogr.*, 2 (1979) 1205.
8 B. B. Jones, B. C. Clark, Jr., and G. A. Iacobucci, *J. Chromatogr.*, 178 (1979) 575.
9 B. Coq, G. Cretier, C. Gonnet and J. L. Rocca, *Chromatographia*, 12 (1979) 139.
10 T. Yoshida, C. Shu and E. T. Theimer, *J. Chromatogr.*, 137 (1977) 461.
11 R. G. Berg and H. M. McNair, *J. Chromatogr.*, 131 (1977) 185.
12 J. Krupčík, J. Kříž, D. Prušová, P. Suchánek and Z. Čerenka, *J. Chromatogr.*, 142 (1977) 797.
13 E. Godbille and P. Devaux, *J. Chromatogr. Sci.*, 12 (1974) 564.
14 E. Godbille and P. Devaux, *J. Chromatogr.*, 122 (1976) 317.
15 C. Djerassi, G. Rosenkranz, J. Romo, S. Kaufmann and J. Pataki, *J. Amer. Chem. Soc.*, 72 (1950) 4534.
16 J. A. Marshall, R. A. Ruden and L. K. Hirsch, *Tetrahedron Lett.*, (1971) 3795.
17 E. J. Corey and R. H. K. Chen, *Tetrahedron Lett.*, (1973) 1611.
18 J. A. Campbell and J. C. Babcock, *J. Amer. Chem. Soc.*, 81 (1959) 4069.
19 H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, 91 (1969) 7445.
20 J. Montastier, S. Cacchi, F. Gasparrini, D. Misiti, L. Charles, J. Giglio and L. Caglioti, *Pittsburg Conference, Cleveland, Ohio, U.S.A., 5 March 1979.*
21 J. Montastier, L. Charles, J. Giglio, L. Caglioti, F. Gasparrini, M. Giovannoli and D. Misiti, *Pittsburg Conference, Atlantic City, NJ, U.S.A., March 1980.*