MASS SPECTRA OF 12-OXOLUPANE DERIVATIVES*

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The mass spectra of a series of substituted 12-oxolupane derivatives *I*—*XIV* and *XVII* were studied. The basic type of molecular ion fragmentation is a retro-Diels–Alder cleavage of the enol form occurring in ring C, under formation of fragments of type *a*. To a lesser extent ions of type *b* are also formed. The ions formed are characteristic of lupane derivatives with an oxo group in the position 12; hence, they may serve for the detection of this oxo group. The composition of ions was proved by high resolution MS and the fragmentation schemes were proved by means of the technique of direct analysis of daughter ions. On the basis of the facts available it was proved that the second hydroxy group, or the oxo group of the natural lupane derivative — thurberine or thurberodione is not in the position 12 as originally supposed.

As the mass spectra of the 12-oxolupane derivatives prepared by us did not agree with those of the supposed 12-lupanone derivatives isolated from natural materials[1,2], we decided to investigate their fragmentation and explain the differences observed. A series of 12-oxolupane derivatives with various substituents was selected: 28-hydroxy-12-lupanone[3] (*I*), 13β,28-dihydroxy-12-lupanone (*II*), 3β,28-diacetoxy-12-lupanone (*III*), 3β,28-diacetoxy-13β-hydroxy-12-lupanone (*IV*), (20*S*)-20-acetoxy-30-nor-12-lupanone[4] (*V*), (20*S*)-3β,20,28-triacetoxy-30-nor-12-lupanone[4] (*VI*), 3β,20,28-triacetoxy-29,30-dinor-12-lupanone[5] (*VII*), 3β,28-diacetoxy-12-oxo-29,30-dinorlupan-20-oic acid[6] (*VIII*), methyl 3β,28-diacetoxy-12-oxo-29,30-dinorlupan-20-oate[6] (*IX*), 3β,28-dimethoxy-30-nor-12,20-lupanedione[7] (*X*), (20*S*)-3β,28-dimethoxy-20-hydroxy-30-nor-12-lupanone[7] (*XI*), (20*S*)-3β,28-dimethoxy-20-acetoxy-30-nor-12-lupanone[7] (*XII*), 3β,28-dimethoxy-20-hydroxy-29,30-dinor-12-lupanone[7] (*XIII*), 3β,28-dimethoxy-20-acetoxy-29,30-dinor-12-lupanone[7] (*XIV*) and methyl 28-acetoxy-3,12-dioxo-29,30-dinorlupan-20-oate (*XVII*). Hydroxy derivative *II* has been obtained[3] as a by-product during the synthesis of derivative *I*. Derivatives *III* and *IV* were obtained analogously as *I* and *II*, under the effect of hydrogen peroxide in acetic acid on 3β,28-diacetoxy-12-lupene[3] (*XXIII*). No other 12-oxolupane derivative has been synthesized so far and the number of supposed 12-oxolupane derivatives isolated from natural material is also rather limited[1,8,9]. A mass spectrum has been published for thurberodione only[1] (*XVIII*) where the main fragment characterizing the fragmentation was

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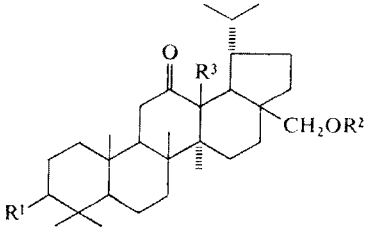
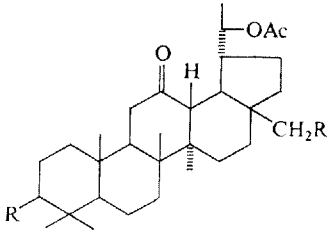
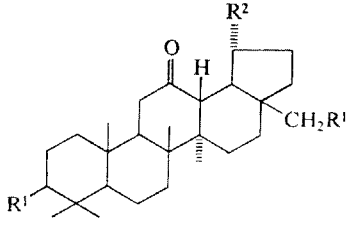
ion *c*, *m/e* 247 (91·5%), from which ions of *m/e* 229 (47·5%), 219 (8%), and 205 (27·5%) were formed after elimination of water, carbon monoxide or ketene. The same fragmentation is also proposed[2] for 3,12,16-trioxo derivative of hopane (*XIX*) even though the intensity of the fragments was low: *m/e* 247 (2%), 229 (2%), 219 (8%). In paper[1] the proof of the position of 12-oxo groups has been carried out only indirectly, and for derivative *XIX* the structure was confirmed[2] by X-ray diffraction method; in neither of the two papers was the composition of the key ions corroborated by high resolution. In contrast to this in the case of 3β-acetoxy-12-oxo-18α-ursane[10] (*XXI*) the fragmentation is quite different. The main fragment here is ion *d*, *m/e* 234 (100%) the formation of which is explained by a formal retro-Diels–Alder cleavage of the enol form in ring C, which is analogous to the cleavage of Δ^{12}-unsaturated derivatives of oleanene[10,11], ursene[10] and lupene[12] derivatives. We would expect a similar type of fragmentation also in 3,12-dioxo-13β,28-epoxyoleanane[13] (*XX*).



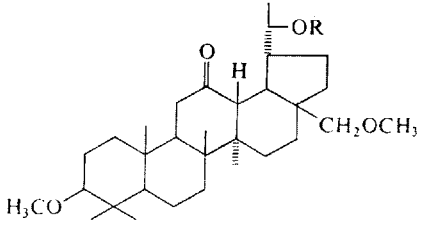
I, $R^1 = R^2 = R^3 = H$
II, $R^1 = R^2 = H$, $R^3 = OH$
III, $R^1 = OAc$, $R^2 = Ac$, $R^3 = H$
IV, $R^1 = OAc$, $R^2 = Ac$, $R^3 = OH$



V, R = H
VI, R = OAc



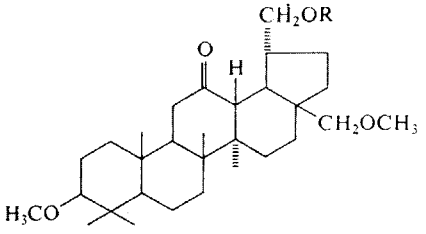
VII, $R^1 = OAc$, $R^2 = CH_2OAc$
VIII, $R^1 = OAc$, $R^2 = COOH$
IX, $R^1 = OAc$, $R^2 = COOCH_3$
X, $R^1 = OCH_3$, $R^2 = COCH_3$



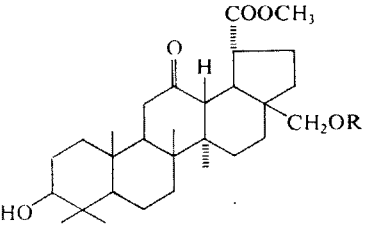
XI, R = H
XII, R = Ac

As the retro-Diels–Alder cleavage of the ring C is so advantageous from the point of view of energy that it remains equal irrelevant of whether the ring E is a five--member or a six-member one or whether the annelation of the rings D/E is *cis* or

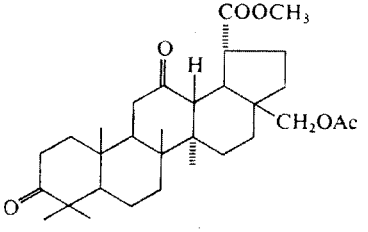
trans, it is improbable that the fragmentation of the lupanone derivative[1] described is ruled by the 12-oxo group and that the fragmentation of the 12-hopanone derivative[2] is the principal one. Another difference between derivatives *XVIII* and *XIX* and *XXI* is in the substitution of the position 3. While derivatives *XVIII* and *XIX* have a keto group in this position, derivative *XXI* has a 3β-acetoxy group in it. Therefore we prepared 3,12-dioxo derivative of lupane *XVII* from the starting acid[6] *VIII*, *via* its methyl ester[6] *IX*, which was hydrolyzed with alkali to diol *XV*. From it partial acetylation[14] gave monoacetate *XVI* which was oxidized with chromium trioxide in pyridine to diketone *XVII*. Its structure was proved by ^{1}H-NMR spectra analysis.



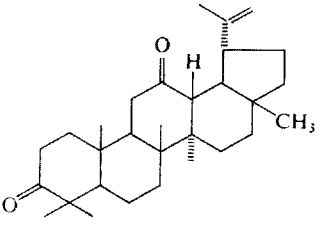
XIII, R = H
XIV, R = Ac



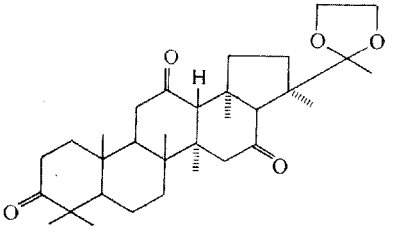
XV, R = H
XVI, R = Ac



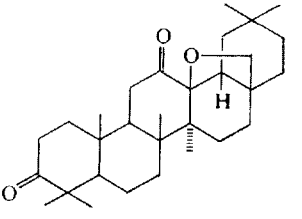
XVII



XVIII

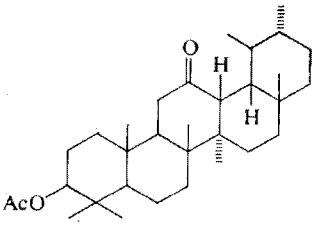


XIX

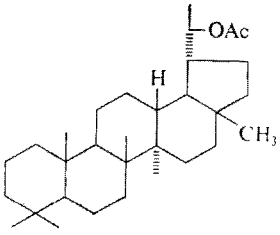


XX

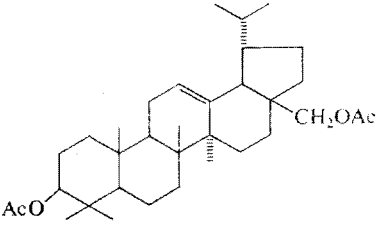
The presence of a 12-oxo group in the molecule is confirmed by the signal of 13β hydrogen (2·64 p.p.m.) which appears in the form of a doublet with $J_{13,18} \approx 11$ Hz, and the position of the 8β-methyl group signal at 1·306 p.p.m. These signals are



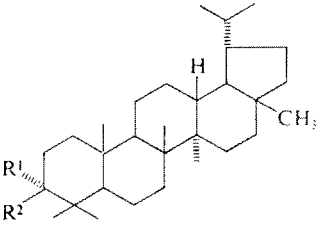
XXI



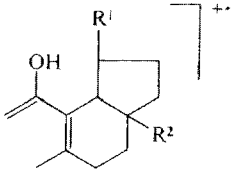
XXII



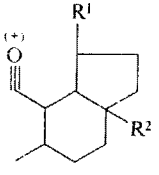
XXIII



XXIV, $R^1 = H$, $R^2 = OAc$
XXV, $R^1 + R^2 = O$



a



b

a_1, *m/e* 250, $R^1 = CH(CH_3)_2$, $R^2 = CH_2OH$
a_2, *m/e* 292, $R^1 = CH(CH_3)_2$, $R^2 = CH_2OAc$
a_3, *m/e* 218, $R^1 = CH{=}CH_2$, $R^2 = CH_3$
a_4, *m/e* 276, $R^1 = CH{=}CH_2$, $R^2 = CH_2OAc$
a_5, *m/e* 248, $R^1 = CH{=}CH_2$, $R^2 = CH_2OCH_3$
a_6, *m/e* 264, $R^1 = COCH_3$, $R^2 = CH_2OCH_3$
a_7, *m/e* 252, $R^1 = CH_2OH$, $R^2 = CH_2OCH_3$
a_8, *m/e* 308, $R^1 = COOCH_3$, $R^2 = CH_2OAc$
a_9, *m/e* 294, $R^1 = COOH$, $R^2 = CH_2OAc$

b_1, *m/e* 205, $R^1 = CH{=}CH_2$, $R^2 = CH_3$
b_2, *m/e* 263, $R^1 = CH{=}CH_2$, $R^2 = CH_2OAc$
b_3, *m/e* 235, $R^1 = CH{=}CH_2$, $R^2 = CH_2OCH_3$
b_4, *m/e* 295, $R^1 = COOCH_3$, $R^2 = CH_2OAc$
b_5, *m/e* 281, $R^1 = COOH$, $R^2 = CH_2OAc$

characteristic of 12-oxolupane derivatives[3-6]. The presence of a 3-oxo group in the molecule follows from the comparison of the methyl signal shifts with those in the spectra of corresponding 3β acetoxy-12-oxolupane derivative *IX*. The values of the

changes agree with the differences between 3β-acetoxylupane (*XXIV*) and 3-oxolupane (*XXV*) (Table I). In the mass spectrum of diketone *XVII* even weak ions at *m/e* 247, 229 and 219 are absent. From this it is evident that the differing fragmentation according to[1] cannot be explained by the effect of the 3-oxo group either. As the only published derivative in which the position of the 12-oxo group is certain is triketone[2] *XIX* (proof by X-ray diffraction)[15], we decided to check its mass spectrum more thoroughly. From Table II it is evident that the ions belonging to the

TABLE I

Characteristic Shifts of Methyl Singlets and Their Changes ($\Delta\delta$) Due to the Anisotropy of Carbonyl

The ^{1}H-NMR spectra were measured in deuteriochloroform, using tetramethylsilane as internal reference. The chemical shifts are given in p.p.m. (δ-scale).

Compound	4α	4β	10β	8β	14α
XXIV	0·84 (0·00)	0·84 (0·00)	0·87 (0·00)	1·04 (0·00)	0·92 (0·00)
XXV[a]	1·08 (+0·24)	1·03 (+0·19)	0·95 (+0·08)	1·07 (+0·03)	0·95 (+0·03)
IX[b]	0·84 (0·00)	0·84 (0·00)	0·89 (0·00)	1·24 (0·00)	0·84 (0·00)
XVII	1·09 (+0·25)	1·06 (+0·22)	1·01 (+0·12)	1·31 (+0·07)	0·88 (+0·04)

[a] The assignment of methyl signals was done according to ref.[16]; [b] See ref.[6].

TABLE II

Mass Spectrum of Triketone *XIX*

The intensity of ions is calculated with reference to ion *m/e* 205, because the intensity of the base peak is too high.

M/e (%, composition): 512 (3·5, $C_{32}H_{48}O_5$), 497 (85, $C_{31}H_{45}O_5$), 453 (19, $C_{29}H_{41}O_4$), 435 (11, $C_{29}H_{39}O_3$), 425 (84, $C_{28}H_{41}O_3$), 411 (10, $C_{27}H_{39}O_3$), 409 (12, $C_{27}H_{37}O_3$), 369 (8, $C_{24}H_{33}O_3$), 302 (8, $C_{20}H_{30}O_2$), 289 (12, $C_{19}H_{29}O_2$), 277 (8, $C_{17}H_{25}O_3$), 249 (23, $C_{15}H_{21}O_3$), 247 (12, d (2 : 1) $C_{15}H_{19}O_3$, $C_{16}H_{23}O_2$), 233 (15, d (2 : 1) $C_{14}H_{17}O_3$, $C_{15}H_{21}O_2$), 231 (15, d (3 : 1) $C_{15}H_{19}O_2$, $C_{16}H_{23}O$), 229 (13, d (2 : 1) $C_{15}H_{17}O_2$, $C_{16}H_{21}O$), 221 (25, d (5 : 1) $C_{14}H_{21}O_2$, $C_{15}H_{25}O$), 219 (26, d (2 : 1) $C_{14}H_{19}O_2$, $C_{15}H_{23}O$), 209 (50, $C_{12}H_{17}O_3$), 205 (100, d (1 : 2) $C_{13}H_{17}O_2$, $C_{14}H_{21}O$), 87 (4250, $C_4H_7O_2$).

TABLE III

Mass Spectra of Derivatives *I*—*XIV* and *XVII*

I, $C_{30}H_{50}O_2$, m.w. 442

m/e (%, composition): 442 (16, $C_{30}H_{50}O_2$), 427 (26, $C_{29}H_{47}O_2$), 411 (14, $C_{29}H_{47}O$), 409 (12, $C_{29}H_{45}O$), 393 (10, $C_{29}H_{45}$), 250 (9·5, $C_{16}H_{26}O_2$), 219 (19, $C_{15}H_{23}O$), 205 (18, $C_{14}H_{21}O$), 201 (19, $C_{15}H_{21}$), 191 (100, d (6 : 1) $C_{14}H_{23}$, $C_{13}H_{19}O$), 177 (80, d (6 : 1) $C_{13}H_{21}$, $C_{12}H_{17}O$).

II, $C_{30}H_{50}O_3$, m.w. 458

m/e (%, composition): 458 (6, $C_{30}H_{50}O_3$), 440 (21, $C_{30}H_{48}O_2$), 427 (7), 425 (6), 422 (6, $C_{30}H_{46}O$), 409 (6, $C_{29}H_{45}O$), 397 (8, $C_{27}H_{41}O_2$), 275 (23, $C_{19}H_{31}O$), 248 (15, $C_{16}H_{24}O_2$), 235 (32, $C_{15}H_{23}O_2$), 223 (11, $C_{14}H_{23}O_2$), 217 (28, $C_{15}H_{21}O$), 207 (33, $C_{14}H_{23}O$), 205 (21, $C_{14}H_{21}O$), 191 (100, $C_{14}H_{23}$), 177 (46, $C_{13}H_{21}$).

III, $C_{34}H_{54}O_5$, m.w. 542

m/e (%): 542 (4), 527 (9), 482 (4), 467 (5), 292 (7), 277 (7), 249 (8), 232 (13), 219 (11), 217 (10), 201 (13), 189 (41), 175 (24), 43 (100).

IV, $C_{34}H_{54}O_6$, m.w. 558

m/e (%): 558 (4), 540 (2), 527 (2), 498 (6), 333 (8), 290 (8), 277 (8), 273 (6), 265 (5), 253 (6), 249 (10), 235 (10), 217 (10), 205 (10), 204 (13), 189 (56), 175 (15), 43 (100).

V, $C_{31}H_{50}O_3$, m.w. 470

m/e (%, composition): 470 (4, $C_{31}H_{50}O_3$), 455 (1, $C_{30}H_{47}O_3$), 427 (100, $C_{29}H_{47}O_2$), 410 (28, $C_{29}H_{46}O$), 395 (27, $C_{28}H_{43}O$), 377 (3, $C_{28}H_{41}$), 218 (34, $C_{15}H_{22}O$), 205 (33, d (3 : 1) $C_{14}H_{21}O$, $C_{15}H_{25}$), 203 (35), 191 (50, $C_{14}H_{23}$), 177 (61, d (1 : 6) $C_{12}H_{17}O$, $C_{13}H_{21}$).

VI, $C_{35}H_{54}O_7$, m.w. 586

m/e (%, composition): 586 (2, $C_{35}H_{54}O_7$), 571 (1, $C_{34}H_{51}O_7$), 543 (27, $C_{33}H_{51}O_6$), 526 (12, $C_{33}H_{50}O_5$), 511 (20, $C_{32}H_{47}O_5$), 483 (16, $C_{31}H_{47}O_4$), 453 (14, $C_{30}H_{45}O_3$), 451 (16, $C_{30}H_{43}O_3$), 276 (18, $C_{17}H_{24}O_3$), 263 (9, $C_{16}H_{23}O_3$), 216 (12), 203 (28, d (3 : 1) $C_{14}H_{19}O$, $C_{15}H_{23}$), 201 (20, d (3 : 1) $C_{14}H_{17}O$, $C_{15}H_{21}$), 43 (100, C_2H_3O).

VII, $C_{34}H_{52}O_7$, m.w. 572

m/e (%, composition): 572 (4, $C_{34}H_{52}O_7$), 557 (2, $C_{33}H_{49}O_7$), 529 (6, $C_{32}H_{49}O_6$), 512 (20, $C_{32}H_{48}O_5$), 497 (39, $C_{31}H_{45}O_5$), 469 (8, d (1 : 5) $C_{29}H_{41}O_5$, $C_{30}H_{45}O_4$), 439 (21, $C_{29}H_{43}O_3$), 437 (13, $C_{29}H_{41}O_3$), 267 (35, $C_{15}H_{23}O_4$), 262 (35, $C_{16}H_{22}O_3$), 249 (20, d (2 : 1) $C_{15}H_{21}O_3$, $C_{16}H_{25}O_2$), 220 (14, $C_{14}H_{20}O_2$), 202 (24, d (8 : 1) $C_{14}H_{18}O$, $C_{15}H_{22}$), 189 (78), 175 (41), 43 (100).

VIII, $C_{32}H_{48}O_7$, m.w. 544

m/e (%, composition): 544 (5, $C_{32}H_{48}O_7$), 529 (6), 526 (5), 511 (17), 498 (5, $C_{30}H_{42}O_6$), 484 (8), 469 (15), 466 (10), 425 (14, $C_{28}H_{41}O_3$), 294 (25, $C_{16}H_{22}O_5$), 281 (8), 276 (19, $C_{16}H_{20}O_4$), 249 (10, $C_{16}H_{25}O_2$), 248 (8), 234 (19, $C_{14}H_{18}O_3$), 204 (21), 189 (67), 175 (55), 43 (100).

TABLE III
(Continued)

IX, $C_{33}H_{50}O_7$, m.w. 558

m/e (%): 558 (5), 543 (10), 527 (5), 511 (27), 498 (12), 483 (5), 469 (11), 439 (9), 425 (12), 308 (18), 295 (12), 276 (15), 248 (34), 206 (46), 189 (32), 175 (33), 43 (100).

X, $C_{31}H_{50}O_4$, m.w. 486

m/e (%, composition): 486 (6, $C_{31}H_{50}O_4$), 471 (27, $C_{30}H_{47}O_4$), 453 (6, $C_{30}H_{45}O_3$), 441 (14, $C_{29}H_{45}O_3$), 264 (32, $C_{16}H_{24}O_3$), 246 (19, $C_{16}H_{22}O_2$), 232 (18, $C_{15}H_{20}O_2$), 221 (24, d (6 : 1) $C_{14}H_{21}O_2$, $C_{15}H_{25}O$), 201 (30, d (5 : 1) $C_{14}H_{17}O$, $C_{15}H_{21}$), 189 (50), 175 (25), 43 (100).

XI, $C_{31}H_{52}O_4$, m.w. 488

m/e (%, composition): 488 (10, $C_{31}H_{52}O_4$), 473 (10, $C_{30}H_{49}O_4$), 470 (17, $C_{31}H_{50}O_3$), 455 (34, $C_{30}H_{47}O_3$), 444 (53, $C_{29}H_{48}O_3$), 429 (75, $C_{28}H_{45}O_3$), 425 (50), 393 (13), 238 (5, $C_{19}H_{28}O_2$), 248 (20, $C_{16}H_{24}O_2$), 235 (22, $C_{15}H_{23}O_2$), 221 (57, $C_{15}H_{25}O$), 203 (53, d (3 : 1) $C_{14}H_{19}O$, $C_{15}H_{23}$), 189 (100), 175 (72).

XII, $C_{33}H_{54}O_5$, m.w. 530

m/e (%, composition): 530 (17, $C_{33}H_{54}O_5$), 515 (2), 487 (24), 470 (17), 455 (47), 438 (9, $C_{30}H_{46}O_2$), 425 (64, $C_{29}H_{45}O_2$), 393 (10), 248 (25, $C_{16}H_{24}O_2$), 235 (19, $C_{15}H_{23}O_2$), 221 (25, $C_{15}H_{25}O$), 203 (35, d (6 : 1) $C_{14}H_{19}O$, $C_{15}H_{23}$), 189 (60), 175 (44), 43 (100).

XIII, $C_{30}H_{50}O_4$, m.w. 474

m/e (%, composition): 474 (12, $C_{30}H_{50}O_4$), 459 (45, $C_{29}H_{47}O_4$), 456 (12), 444 (17), 441 (39, $C_{29}H_{45}O_3$), 429 (43, $C_{28}H_{45}O_3$), 411 (47, $C_{28}H_{43}O_2$), 274 (12, $C_{18}H_{26}O_2$), 252 (9, $C_{15}H_{24}O_3$), 234 (17, $C_{15}H_{22}O_2$), 227 (15, d (4 : 1) $C_{16}H_{19}O$, $C_{17}H_{23}$), 221 (53, d (1 : 1) $C_{14}H_{21}O_2$, $C_{15}H_{25}O$), 189 (78, d (1 : 1) $C_{13}H_{17}O$, $C_{14}H_{21}$), 175 (80, d (1 : 1) $C_{12}H_{15}O$, $C_{13}H_{19}$), 147 (100).

XIV, $C_{32}H_{52}O_5$, m.w. 516

m/e (%): 516 (4), 501 (2), 456 (5), 441 (33), 425 (5), 411 (17), 294 (3), 234 (15), 227 (12), 221 (24), 202 (30), 189 (52), 175 (38), 43 (100).

XVII, $C_{31}H_{46}O_6$, m.w. 514

m/e (%, composition): 514 (4, $C_{31}H_{46}O_6$), 499 (7, $C_{30}H_{43}O_6$), 483 (10, $C_{30}H_{43}O_5$), 467 (34, $C_{29}H_{39}O_5$), 454 (7), 439 (3, $C_{28}H_{39}O_4$), 425 (25, $C_{27}H_{37}O_4$), 407 (6, $C_{27}H_{35}O_3$), 394 (9, $C_{27}H_{38}O_2$), 381 (20, $C_{26}H_{37}O_2$), 308 (22, $C_{17}H_{24}O_5$), 295 (10, $C_{16}H_{23}O_5$), 276 (27, $C_{16}H_{20}O_4$), 248 (35, $C_{15}H_{20}O_3$), 235 (14, $C_{14}H_{19}O_3$), 216 (30, $C_{14}H_{16}O_2$), 206 (71, d (3 : 1) $C_{13}H_{18}O_2$, $C_{14}H_{22}O$), 205 (43, d (1 : 2) $C_{13}H_{17}O_2$, $C_{14}H_{21}O$), 147 (100, $C_{11}H_{15}$).

supposed fragmentation of 12-oxo derivatives are of very small intensity. They are doublets in which the ion of the composition corresponding to this fragmentation is always less represented. For example ion *m/e* 247 is composed of a fragment of the compositions $C_{15}H_{19}O_3$ and $C_{16}H_{23}O_2$, which occur in a 2 : 1 ratio. From this we judge that the proposed fragmentation will not be the main type of fragmentation of this derivative. Simultaneously, owing to the oxo group in the position 16, the fragmentation becomes more complex than in the case of our 3,12-dioxo derivative *XVII*, and therefore it will not be typical of the fragmentation of 12-oxo derivatives.

EXPERIMENTAL

The melting points were measured on a Kofler block and they were not corrected. Optical rotation was measured in chloroform on an automatic ETL-NPL (Bendix-Ericsson) polarimeter, with a ±2% accuracy. The infrared spectra were measured in chloroform on a UR 20 (Zeiss, Jena) spectrophotometer. The ^{1}H-NMR spectra were measured in deuteriochloroform, using tetramethylsilane as internal reference, on a Varian HA-100 instrument; chemical shifts are in p.p.m., δ-scale. The mass spectra were measured on a Varian MAT 311 spectrometer, energy of ionizing electrons 70 eV, ionizing current 1 mA, ion source temperature 200°C, temperature of direct inlet system 90—190°C. The composition of all ions in Table III was confirmed by high resolution, with an error of less than 5 p.p.m. All fragmentation transitions were proved by the DADI (direct analysis of daughter ions)[17] technique.

3β,28-Diacetoxy-12-lupanone (*III*) and 3β,28-Diacetoxy-13β-hydroxy-12-lupanone (*IV*)

Hydrogen peroxide (2·5 ml, 30%) in acetic acid (2·5 ml) was added dropwise to a solution of 3β,28-diacetoxy-12-lupene[3] (*XXIII*) (200 mg) in acetic acid (8 ml) and the mixture was heated on a water bath for 3 hours. Hot water was added (20 ml) and the precipitated substance obtained by filtration. Chromatography on silica gel (30 g), using light petroleum for elution, gave 50 mg of 12-oxo derivative *III*, m.p. 245—248°C (ether–hexane), $[\alpha]_D$ −10° (*c* 0·20), IR spectrum: 1030, 1255, 1723 (CH_3COO), 1709 (C=O) cm^{-1}. For $C_{34}H_{54}O_5$ (542·9) calculated: 75·23% C, 10·03% H; found: 75·65% C, 9·76% H. On elution with a mixture of light petroleum and ether (9 : 1) 40 mg of hydroxy derivative *IV* were obtained, m.p. 279—283°C (ether), $[\alpha]_D$ −4° (*c* 0·49), IR spectrum: 1030, 1255, 1724 (CH_3COO), 1710 (C=O), 3600 (OH) cm^{-1}.

Methyl 28-Acetoxy-3,12-dioxo-29,30-dinorlupan-20-oate (*XVII*)

A solution of potassium hydroxide (200 mg) in ethanol (25 ml) was added to a solution of methyl ester[6] *IX* (310 mg) in benzene (30 ml) and the mixture was heated at 50°C for 2 hours. After evaporation of solvent the residue was dissolved in ether and washed with 5% hydrochloric acid and water, dried over anhydrous sodium sulfate and evaporated. Yield 220 mg of amorphous diol *XV*. IR spectrum: 1430, 1714 ($COOCH_3$), 1700, (C=O), 3610 (OH) cm^{-1}. Acetic anhydride (0·5 ml) was then added dropwise to a solution of diol *XV* (210 mg) in pyridine (10 ml) at 0°C. The mixture was allowed to stand in the cold for 40 minutes, then decomposed with water and extracted with ether. The ethereal fraction was extracted with water, dried over anhydrous sodium sulfate and evaporated. Yield 170 mg of a mixture of monoacetate *XVI* with starting diol *XV*. After separation of the mixture by preparative thin-layer chromatography

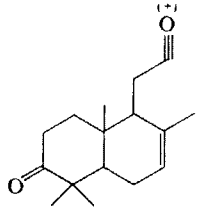
on silica gel 100 mg of monoacetate *XVI* and 20 mg of diol *XV* were obtained. IR spectrum of monoacetate *XVI*: 1035, 1240, 1720 (CH_3COO), 1700 (C=O), 1430, 1720 ($COOCH_3$), 3600 (OH) cm^{-1}. Chromium trioxide (175 mg) dissolved in pyridine (2 ml) was then added dropwise to a solution of monoacetate *XVI* (70 mg) in pyridine (3 ml) and the mixture was allowed to stand at room temperature for 2 hours. After dilution with water it was extracted with ether and the extract washed three times with hydrochloric acid (5%) and with water, dried over anhydrous sodium sulfate and evaporated. Yield 60 mg of amorphous diketone *XVII*. IR spectrum: 1035, 1240, 1720 (CH_3COO), 1700 (C=O), 1720 ($COOCH_3$) cm^{-1}. ^{1}H-NMR spectrum: 0·88 s (CH_3), 1·01 s (CH_3), 1·06 s (CH_3), 1·09 s (CH_3), 1·31 s (CH_3), 2·07 s (28--CH_3COO), 2·3 m ($C_{(11)}$—H_2, $C_{(19)}$—H, $C_{(2)}$—H_2), 2·63 d, J = 11 Hz ($C_{(13)}$—H), 3·63 d + + 4·23 d ($C_{(28)}$—H_2), 3·75 s ($COOCH_3$) p.p.m. CD spectrum (dioxan): λ_{max} ($\Delta\varepsilon$) = 323 (0), 312 (+0·17) 307 (+0·08), 302 (+0·15), 297 (0), 277 (−0·17), 252 (0).

RESULTS AND DISCUSSION

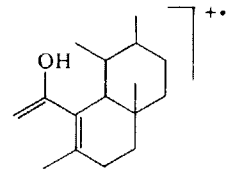
Derivatives *I*–*XIV* and *XVII* were divided into 5 groups according to structural relationships and thus also similar fragmentation pattern.

Group *A* includes the simplest 12-oxolupane derivatives *I* and *III*. Molecular ion of 28-hydroxy-12-lupanone (*I*) loses a methyl radical under formation of ion *m/e* 427, radical CH_2OH under formation of ion *m/e* 411, and ion a_1, *m/e* 250, is formed by retro-Diels–Alder cleavage of ring C (by a formally explainable formation of enol form). The ion *m/e* 427 loses a molecule of water under formation of ion *m/e* 409. Ion *m/e* 411 also loses a molecule of water under formation of ion *m/e* 393, while ion e_1, *m/e* 219, is formed by cleavage of ring C (analogously as in the case of ion *m/e* 442). Further ions *m/e* 205, 191 (f_1) (ref.[10]), and 177 (g_1) are formed. Ions of type *f* and *g* represent the fragments of rings A and B. In both cases their formation is explicable by retro-Diels–Alder decomposition of the enol form which does not take place in a simple manner but under transfer of hydrogen[10] from the methyl group 8β or by migration or splitting of a methyl radical. Ion e_1 is also formed from ion a_1 by loss of radical $^{\bullet}CH_2OH$ which sets a molecule of water free under formation of ion *m/e* 201. Diacetate *III* is fragmented analogously. Fragment a_2, *m/e* 292, liberates the radical $^{\bullet}CH_2OAc$ under formation of ion e_1, *m/e* 219. Fragments f_2, *m/e* 249, *h*, *m/e* 189 (ref.[10]), and *i*, *m/e* 175 belong to rings A and B. In both substances the ions of type *b* are of negligible intensity.

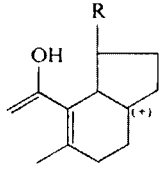
Substances of group *B* (*V*–*VII*) have an acetoxyl group in the position 20 and they differ in the substitution at the positions 3β,20 and 28. The first fragment which occurs in all three substances is formed on loss of a methyl radical and it is of very low intensity. The further, common loss is 43 mass units (C_2H_3O), occurring in these three substances only and in derivative *XII*, *i.e.* only in substances with an acetoxyl group in the position 20. The ion formed from derivative *V* is even the base peak. Its occurrence could be explained either by a simultaneous loss of carbon monoxide and a methyl radical, or – more probably – by the loss of the radical $CH_3CO^{\bullet}$ from the acetoxy group in the position 20. That this radical does not originate from another



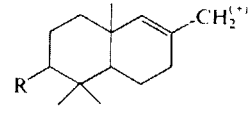
c, *m/e* 247



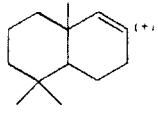
d, *m/e* 234



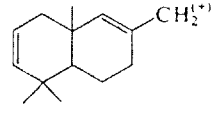
e_1, *m/e* 219, R = $CH(CH_3)_2$
e_2, *m/e* 203, R = $CH{=}CH_2$



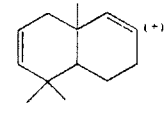
f_1, *m/e* 191, R = H
f_2, *m/e* 249, R = OAc
f_3, *m/e* 221, R = OCH_3



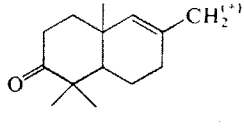
g, *m/e* 177



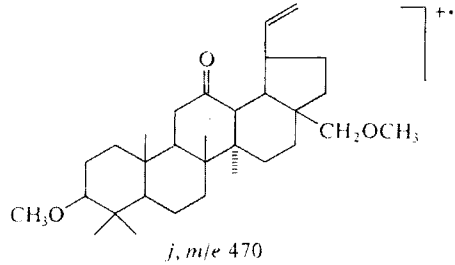
h, *m/e* 189



i, *m/e* 175



k, *m/e* 205

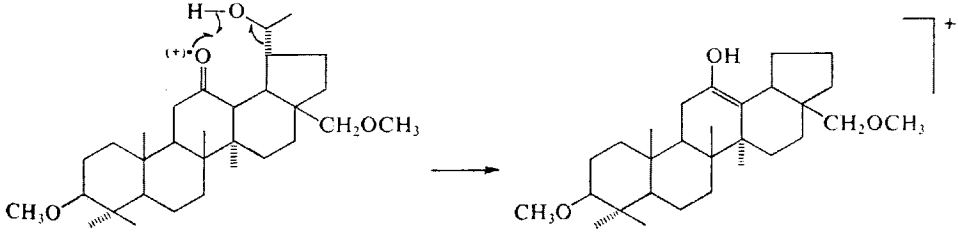


j, *m/e* 470

acetoxy group is proved by the ion *m/e* 427 in derivative *V* in which there is no other acetoxy group. This cleavage takes place in the presence of 12-oxo group only, because in derivative *XXII* which differs from *V* by the absence of the 12-oxo group only, the loss of 43 mass units simply does not occur. Thus, ions of *m/e* 455, 427 originate from the molecular ion of *V*, and the ion *m/e* 410 is formed by the loss of one molecule of acetic acid according to the McLafferty rearrangement. The latter ion loses a methyl radical under formation of ion *m/e* 395; ion a_3, *m/e* 218, is formed by retro-Diels–Alder cleavage of the ring C (similarly as in group *A*), ion f_1 is formed

by an analogous cleavage and the ion b_1 of *m/e* 205 is formed by α-cleavage (with respect to the carbonyl group) connected with hydrogen transfer. Ion a_3 loses a methyl radical under formation of ion *m/e* 203. Fragmentation of derivatives *VI* and *VII* is analogous. In derivative *VI* ions a_4, *m/e* 276, and b_2, *m/e* 263 are formed. From ion a_4 the ion e_2 of *m/e* 203 is formed by loss of radical $^{\bullet}CH_2OAc$. In derivative *VII* the analogous ions of type *a* and *b* occur at *m/e* 262 and 249.

3β,28-Dimethoxy derivatives were classified into group *C* (substances *X*–*XIV*), differing by substitution in the position 20. The molecular ion of derivative *XII*, *m/e* 530, is fragmented in two ways. It loses the radical $CH_3CO^{\bullet}$ from the acetyl group in the position 20 in the same way as substances from group *B*. The ion *m/e* 487 loses a molecule of methanol from ring A, under formation of ion *m/e* 455. The second type of cleavage of the molecular ion consists in the loss of a molecule of acetic acid from the side chain, in the position 19α, by McLafferty's rearrangement, under formation of ion *j*, *m/e* 470, which is further split in four ways. It loses a molecule of methanol from the ring A under formation of ion *m/e* 438, it loses the radical $^{\bullet}CH_2OCH_3$ under formation of ion *m/e* 425, the base fragment a_5 of *m/e* 248 is formed by retro-Diels–Alder cleavage of the enol form, and the ion b_3 with *m/e* 235 is formed by α-cleavage (with respect to the carbonyl group in ring C) connected with hydrogen transfer. The ion *m/e* 425 is further fragmented. The loss of a molecule of methanol from ring A brings about the formation of ion *m/e* 393, and retro-Diels–Alder cleavage of the enol form of ring C produces ion f_3, *m/e* 221, and ion e_2 of *m/e* 203 is formed analogously after charge transfer. If the loss of a methanol molecule takes place simultaneously, instead of ion f_3 ion *h* is formed. From ion a_5 ion e_2 is also formed by a loss of radical $^{\bullet}CH_2OCH_3$. Derivative *XI* is cleaved in an analogous manner. The loss of the molecule of acetaldehyde from the side chain in the position 19α under formation of ion *m/e* 444 is interesting here. This ion evidently has an enolate structure formed by additional migration of the hydrogen from position 13β to position 19 (Scheme 1). Ion *m/e* 429 is formed by simultaneous loss of acetaldehyde and methyl radical. Derivative *X* is again cleaved analogously. The



m/e 488 *m/e* 444

SCHEME 1

base fragment a_6 of *m/e* 264 loses a molecule of water under formation of ion *m/e* 246, it then loses the radical $CH_3CO^{\bullet}$ under formation of ion *m/e* 221, and a simultaneous loss of a water molecule and radical $^{\bullet}CH_2OCH_3$ leads to formation of ion *m/e* 201. In the mass spectra of derivatives *XIII* and *XIV* which differ from the preceding ones in this group only by the fact that they do not have a methyl group in the position 20, the expected fragments again appear. Thus, in the spectrum of derivative *XIII* ion a_7 is present (*m/e* 252) which liberates a molecule of water under formation of ion *m/e* 234, or it splits the radical $^{\bullet}CH_2OH$ under formation of ion *m/e* 221. Entirely analogous fragments are also found in the case of derivative *XIV*.

12-oxo derivatives substituted in the position 19α with a carboxyl or methoxycarbonyl group were classified into the group *D* (substances *VIII*, *IX* and *XVII*). From diketone *XVII* the fragments of higher masses are formed by loss of methyl radical, radical $CH_3O^{\bullet}$, a molecule of methanol, radical $CH_3CO^{\bullet}$, methyl formate molecule, or their combination. The molecular ion is fragmented in two ways. By retro-Diels–Alder cleavage of the enol form ion a_8 of *m/e* 308 is formed, from which ion *m/e* 276 is formed by the loss of a molecule of methanol, and ion *m/e* 248 is formed by the loss of one molecule of methyl formate from the ester group. α-Cleavage (with respect to carbonyl) combined with hydrogen transfer gives rise to ion b_4, *m/e* 295, which after the loss of methyl formate molecule gives ion *m/e* 235. Ion *m/e* 276 loses a molecule of acetic acid under formation of ion *m/e* 216 which is also formed from ion *m/e* 248 by the loss of a molecule of methanol. The ion *m/e* 248 loses the radical $CH_3CO^{\bullet}$ under formation of ion *m/e* 205. A fragment of the same mass is also the known[10] ion *k*, belonging to rings A and B. Ion *m/e* 206 is generated from ion a_8 by the loss of radical $^{\bullet}COOCH_3$ and $^{\bullet}COCH_3$. Derivative *IX* is fragmented in an analogous manner. It differs from the preceding one only in the substituent at position 3. All fragments belonging to rings C, D and E are identical in derivatives *XVII* and *IX*. In the mass spectrum of acid *VIII* fragments were again found which are the consequence of both basic types of fragmentation in this series. In addition to ion a_9, *m/e* 294, which loses a molecule of water under formation of ion *m/e* 276, the ion b_5, *m/e* 281, also occurs.

In group *E* (substances *II* and *IV*) the fragmentation differs from those of the preceding derivatives, due to the effect of the new hydroxy group in the position 13β. From the molecular ion both derivatives lose first a molecule of water, which stems evidently from the new hydroxy group. Further fragmentation is more complex. Only ions belonging to rings A and B (*i.e.* f_1 and *g* in derivative *II* and *h* and *i* in derivative *IV*) can be identified easily.

From the mass spectra of 12-oxolupane derivatives *I*, *III*, *V*–*XIV* and *XVII* it is evident that their fragmentation takes place by two basic routes. The first is a retro-Diels–Alder cleavage of the enol form created in ring C, under formation of ion-radicals of type *a*. The second type is the α-cleavage (with respect to the carbonyl group) in ring C, combined with a hydrogen transfer, under formation of ions of

type *b*. The fragments of type *b* are always less abundant than the fragments of type *a*. The fragmentation of 12-oxolupane derivatives can be considered as unambiguous and quite characteristic of the 12-oxo group. Hence, mass spectrometry may serve for the proof of the 12-oxo group on the lupane skeleton (so long as no other oxo group – except the 3-oxo group – is present on the skeleton, or some other substituent on ring C). The fragmentation of 12-oxolupane derivatives is analogous to that of 12-oxo-18β-ursane derivatives[10]. In the spectra of none of the substances of our whole series could any sign of fragmentation be observed, which was claimed[1] for the supposed 12-oxolupane derivative *XVIII*. For this reason we judge that the second group in thurberine[1], or the second oxo group in thurberodione is not in the position 12.

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