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TRITERPENOIDS—IV[1]

THE CYCLAMIGENINS A^1, A^2, C, AND D

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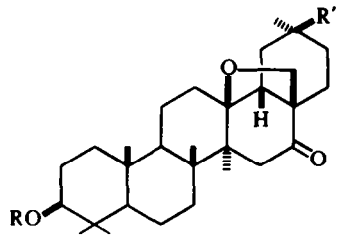
Abstract—The cyclamigenins A^1 and C are 30β- (IXa) and 30α- (IXb) ethoxy-28,30-epoxyolean-12-en-3β,16α-diol, respectively. Cyclamigenin A^2 is 30(or 29)-noraegicerin (V) and cyclamigenin D is 30,30-dimethoxyaegicerin (VII). The identifications are based mainly on spectroscopic data but cyclamigenins A^1 and C have been converted to cyclamiretin D (XI) and cyclamigenin D has been converted to 3β,28-diacetoxy-16-keto-olean-12-en-30-oic acid (XV).

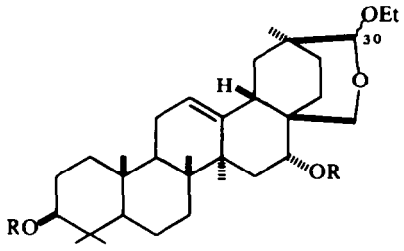
IN A previous communication[1] we described the isolation of the cyclamigenins A, B (I), C, D, and E from the amorphous saponin of *Cyclamen europaeum* corms. A sixth triterpene, which is intermediate in chromatographic mobility between cyclamigenins A and B, has now been isolated. The new cyclamigenin is designated A^2, the original A now being A^1. This paper describes the determination of the structures of cyclamigenins A^1, A^2, C, and D.

Cyclamigenins A^1 and C are isomers, the mass spectra of which show molecular ions at *m/e* 500 ($C_{32}H_{52}O_4$). They are stable to alkali and their IR spectra, which are almost identical, show no carbonyl bands but strong absorption at 3400 (O—H stretch) and 1000 cm^{-1} (C—O stretch) and the group of peaks at ca. 820 cm^{-1} characteristic[2] of the 12(13)-double bond in pentacyclic triterpenes. Both compounds show only end-absorption in the UV. Two OH groups are present in each compound, as is demonstrated by the ready formation of diacetates the mass spectra of which exhibit molecular ions at *m/e* 584 ($C_{36}H_{56}O_6$). That both of the remaining O atoms in cyclamigenins A^1 and C are present in ether functions is indicated by the absence of O—H stretching vibrations in the IR spectra of the acetates. A Zeisel estimation shows that one of these ether functions is an EtO group.

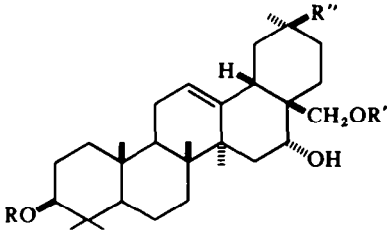
The most obvious structures for cyclamigenins A^1 and C are the 30-epimeric, mixed acetals (IX) of cyclamiretin D (XI).[3] The NMR spectra of the acetates (X) are consistent with this hypothesis in that they show the presence of six angular Me groups; an EtO group as a triplet at ca. τ 8·75, partly obscured by the methylene envelope and an angular Me signal, and a quartet at ca. τ 6·45, partly obscured by the 28-methylene quartet centred at ca. τ 6·5 ($J = -12$ Hz); the H-16β multiplet at ca. τ 6·4; the H-12 multiplet at τ 4·65; and H-30 as a singlet at τ 5·6. Confirmation of these structures was obtained by mild acid-hydrolysis of the acetates both of which yield cyclamiretin D monoacetate (XII), identical with a sample prepared by a similar hydrolysis of cyclamiretin D diacetate (XIII).[3,4]



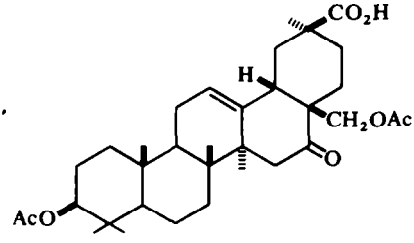
I: R = H, R′ = CHO
II: R = Ac, R′ = CHO
III: R = H, R′ = Me
IV: R = Ac, R′ = Me
V: R = R′ = H
VI: R = Ac, R′ = H
VII: R = H, R′ = $CH(OMe)_2$
VIII: R = Ac, R′ = $CH(OMe)_2$



IXa: R = H, 30β
IXb: R = H, 30α
Xa: R = Ac, 30β
Xb: R = Ac, 30α



XI: R = R′ = H, R″ = CHO
XII: R = Ac, R′ = H, R″ = CHO
XIII: R = R′ = Ac, R″ = CHO
XIV: R = R′ = H, R″ = $CH(OEt)_2$



XV

Ring E is necessarily a twist-boat in cyclamigenins A[1] and C, while the ease of acetylation of the 16α-OH group and the unusually high-field position of the H-16β resonance (τ 6·4) in the NMR spectra of the acetates (i.e. H-16β lies above the double bond) indicate that ring D is a boat with C-13 and C-16 bow and stern. Of the two possible conformations for the oxide bridge the *endo* form (Fig. 1) must be favoured as non-bonding interactions are much less severe than in the *exo* form (Fig. 2). Thus, in the *exo* form (Fig. 2) H-16β and H-28β, AcO-16α and H-28α, and Me-20 and EtO-30α (or H-30α) are eclipsed and in the axial epimer the oxygen of the EtO group is close to H-18 (1·5 Å from Dreiding models). In the *endo* form (Fig. 1) all of

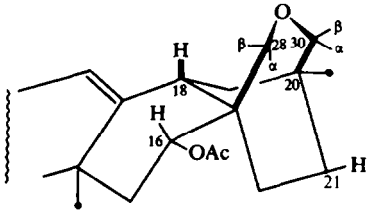


Fig. 1

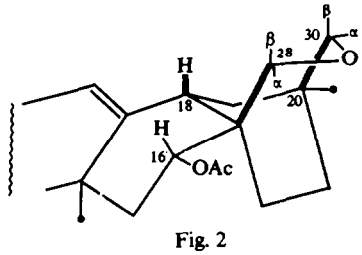
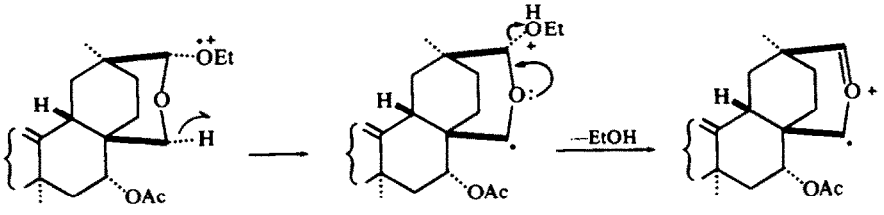


Fig. 2

these bonds are staggered and an axial 30α-EtO group is relatively unhindered (the nearest non-bonded neighbour is H-21β at 2·2 Å). Since the ratio of cyclamigenins A^1:C is ca. 30:1, it is reasonable to assume that cyclamigenin C is the axial, 30α-epimer (IXb) and that cyclamigenin A^1 is the equatorial, 30β-isomer (IXa). There is some slight spectroscopic evidence in favour of these assignments. Thus, in the mass spectrum of cyclamigenin C diacetate M − EtOH(*a*) accounts for the base peak whereas this species is much less abundant (13% relative intensity) in the case of cyclamigenin A^1 diacetate. It seems plausible that such a fragmentation would occur more easily for the axial, 30α-isomer where a preliminary hydrogen-transfer could lead to the expulsion of an ethanol molecule:



Xb *a*

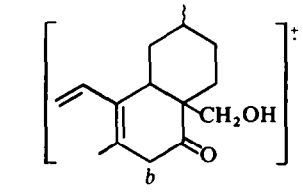
The hydrogen-transfer step is not possible in the case of the equatorial, 30β-epimer. Cyclamigenins A^1 and C are, therefore, 30β- and 30α-ethoxy-28,30-epoxyolean-12-en-3β,16α-diol, respectively.

Both compounds are obviously artifacts although we have not been able to prepare them from cyclamiretin D (XI) which is unchanged when subjected to the conditions of the hydrolysis stage of the extraction,[1] *viz* treatment with aqueous ethanolic hydrochloric acid. With ethanolic hydrogen chloride cyclamiretin D yields the diethyl acetal (XIV).

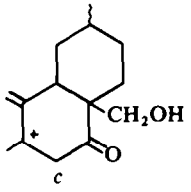
Cyclamigenin A^2 is saturated to tetranitromethane and shows no selective absorption in the UV. Its IR spectrum, which is very similar to that of aegicerin (III),[5] shows the presence of OH (3400 cm^{-1}), ketonic CO (1704 cm^{-1}), and ether oxygen (1028 and 890 cm^{-1}). It forms a monoacetate the NMR spectrum of which is similar to that of aegicerin acetate (IV) (e.g. the methylene group on the ether bridge appears as an AB quartet, $J = -8$ Hz, centred at τ 6·28) except that it indicates the presence of only six skeletal Me groups one of which is secondary (doublet at τ 9·08, $J = 7$ Hz). Cyclamigenin A^2, therefore, is 30(or 29)-noraegicerin (V). Confirmation of this structure is found in the mass spectrum of the acetate (VI) which is very similar to that[1] of aegicerin acetate (IV) except that the fragments containing ring E occur at 14 a.m.u. lower. Thus, the molecular ion appears at *m/e* 484, $C_{31}H_{48}O_4$, and the D/E-ring fragments *b*, *c*, and *d* occur at *m/e* 234, 221, and 189 (base peak), respectively.

Cyclamigenin D, which shows no selective absorption in the UV, absorbs in the IR at 3500 (O—H stretch) and 1707 cm^{-1} (ketonic C=O stretch). The IR spectra of both the alcohol and its acetate show a group of ether bands[6] near 1100 cm^{-1} and one at 890 cm^{-1} (tertiary ether[7]) together with a peak at 2825 cm^{-1} indicative[8] of the presence of MeO. The presence of the latter is confirmed by the NMR spectrum of cyclamigenin D acetate which shows a strong signal at τ 6·52 the integrated intensity of which corresponds to two MeO groups. The spectrum shows only six angular Me groups. A singlet at τ 5·76 (O—C<u>H</u>—O), an AB quartet ($J = -8$ Hz) centred at τ 6·3 (C<u>H</u>$_2$O), and a doublet ($J = -16$ Hz) at τ 7·3 (one proton of C<u>H</u>$_2$CO) suggested

that cyclamigenin D is the dimethyl acetal (VII) of cyclamigenin B (I). The mass spectra of cyclamigenin D (VII) and its acetate (VIII) are consistent with the proposed structure in that the molecular ions occur at *m/e* 516, $C_{32}H_{52}O_5$, and 558, $C_{34}H_{54}O_6$, respectively; A/B-ring fragments[9] are observed at *m/e* 249 (*e*), 207 (*f*), and 189 (*g*); and D/E-ring fragments[9] (e.g. the retro-diene species *h*) carry the acetal group. The skeletal fragments are all of low abundance and ca. 30% of the total ion current in both spectra is carried by the expected[10] α-cleavage species, MeOCH: $\overset{+}{O}Me$, of *m/e* 75.



b



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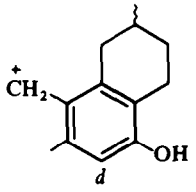
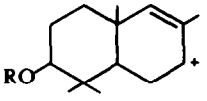
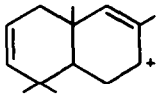


d



e: R = Ac
f: R = H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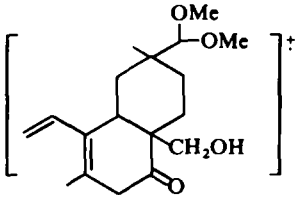

h

Acid hydrolysis of cyclamigenin D yields a mixture of products (presumably liberation of the aldehyde is accompanied by some cleavage of the ether ring and elimination of C-28 as formaldehyde) but acetolysis followed by mild, chromic acid oxidation proceeds smoothly to give 3β,28-diacetoxy-16-ketoolean-12-en-30-oic acid (XV), identical with a sample prepared[3] from cyclamiretin D diacetate (XIII). Finally, brief treatment of cyclamigenin B acetate (II)[1] with anhydrous methanol containing boron trifluoride etherate affords, in poor yield, the dimethyl acetal (VIII) which is identical with cyclamigenin D acetate.

Cyclamigenin D seems obviously an artifact although the only contact between cyclamigenin B and methanol was during crystallization of the acetate (II) in the presence of chloroform and no trace of cyclamigenin D acetate was detected (TLC) after prolonged treatment of the acetate (II) with refluxing chloroform–methanol.

Cyclamigenin E, only trace amounts of which were isolated,[1] was not obtained pure and was not investigated.

EXPERIMENTAL

Specific rotations were determined for 1% solns in $CHCl_3$ at 31° with a Perkin–Elmer 141 Polarimeter. M.ps were determined in vacuum-sealed capillaries in an electrically heated aluminium block and are corrected. IR spectra were recorded for KBr discs on a Grubb–Parsons 'Spectromaster', NMR spectra in $CDCl_3$ on a Varian HR-60A instrument with TMS as internal standard, and mass spectra on A.E.I. MS-9 instruments operating at 70 eV with direct-insertion probes; ion source temp 190–230°. The constitutions of all important ions were confirmed by high-resolution measurements. Analytical samples were dried for 60 hr at 78°/10^{-2} mm.

Cyclamigenin A[1] (IXa)

Cyclamigenin A[1] diacetate, isolated as described previously,[1] had m.p. 231–233°, $[\alpha]_D$ +15 ± 1°, ν_{max} 1735(s), 1235(s), 1000(s), 826(m), 814(w), 797(w) cm^{-1}, ν 9·12 (Me), 9·08 (4Me), 8·79 (tr, J = 7 Hz, Me), 8·77 (Me), 8·01 (Ac), 7·97 (Ac), 6·77 (d, J = −12 Hz, H-28), 6·42 (d, J = −12 Hz, H-28), 6·41 (m, H-16), 6·40 (q, $OC\underline{H}_2Me$), 5·63 (s, H-30), 5·55 (m, H-3), 4·65 (m, H-12). (Found: C, 74·3; H, 9·7; EtO, 7·7. Calc. for $C_{36}H_{56}O_6$: C, 73·9; H, 9·65; EtO, 7·7%). Mass spectrum (*m/e*)/% relative abundance 584 (M^+)/1, 539/4, 538/13, 392/100, 377/50, 334/1, 321/1, 288/3, 274/3, 261/2, 249/2, 228/4, 189/4. Saponification of the acetate with 5% methanolic KOH furnished *cyclamigenin A*[1] as platelets, m.p. 216–217° (from MeOH), $[\alpha]_D$ +42°, ν_{max} 3401(s), 1000(s), 831(m), 817(w), 802(w) cm^{-1}. (Found: C, 75·4; H, 10·5. Calc. for $C_{32}H_{52}O_4 . O . 5MeOH$: C, 75·5; H, 10·5%). Cyclamigenin A[1] is recovered quantitatively after 24 hr in refluxing 10% methanolic KOH. Mass spectrum (*m/e*)/% relative abundance 500 (M^+)/1, 482/2, 455/12, 454/31, 357/27, 292/3, 246/12, 207/17, 189/8, 112/100.

Cyclamigenin C (IXb)

Cyclamigenin C diacetate, isolated as described previously,[1] had m.p. 222–223°, $[\alpha]_D$ −125 ± 2°, ν_{max} 1735(s), 1235(s), 1000(s), 827(m), 814(w), 798(w) cm^{-1}, τ 9·14 (3Me), 9·08 (2Me), 8·81 (tr, J = 7 Hz, Me), 8·74 (Me), 8·01 (Ac), 7·95 (Ac), 6·79 (d, J = −12 Hz, H-28), 6·28 (d, J = −12 Hz, H-28), 6·50 (q, $OC\underline{H}_2Me$), 6·4 (m, H-16), 5·63 (s, H-30), 5·55 (m, H-3), 4·65 (m, H-12). (Found: C, 74·1; H, 9·8; EtO, 8·2. Calc. for $C_{36}H_{56}O_6$: C, 73·9; H, 9·65; EtO, 7·7%). Mass spectrum (*m/e*)/% relative abundance 584 (M^+)/2, 539/40, 538/100, 321/1, 288/7, 274/7, 261/1, 249/2, 228/6, 189/5. Saponification of the acetate with 5% methanolic KOH gave *cyclamigenin C* as platelets, m.p. 218–219° (from MeOH aq), $[\alpha]_D$ −114°, ν_{max} 3401(s), 998(s), 830(m), 815(w), 800(w) cm^{-1}. (Found: C, 76·5; H, 10·3. Calc. for $C_{32}H_{52}O_4$: C, 76·75; H, 10·45%). Cyclamigenin C is recovered quantitatively after 24 hr in refluxing 10% methanolic KOH. Mass spectrum (*m/e*)/% relative abundance 500 (M^+)/2, 482/3, 455/14, 454/27, 357/79, 292/4, 246/8, 207/16, 189/7, 112/100.

Cyclamiretin D monoacetate (XII)

(a) A soln of cylamigenin A[1] diacetate (27 mg) in THF (10 ml) containing 10% HCl aq (3 ml) was left overnight at room temp and then evaporated to dryness at 30°. Crystallization of the residue from $CHCl_3$—MeOH yielded *cyclamiretin D monoacetate* (12 mg), m.p. 237–239°, $[\alpha]_D$ +36°, ν_{max} 3580(s), 2702(w), 1730(s, br), 1245(s) cm^{-1}, identical (mixed m.p. and IR spectrum) with the samples prepared from cyclamigenin C and cyclamiretin D acetates. (Found: C, 72·1; H, 10·1. Calc. for $C_{32}H_{50}O_5 . MeOH$: C, 72·5; H, 10·0%).

(b) Similar treatment of cyclamigenin C diacetate (25 mg) gave cyclamiretin D monoacetate (9 mg), m.p. 237–239°, $[\alpha]_D$ +34°, identical (mixed m.p. and IR spectrum) with samples prepared from cyclamigenin A[1] and cyclamiretin D diacetates.

(c) Similar treatment of cyclamiretin D diacetate[3,4] (50 mg) afforded cyclamiretin D monoacetate (18 mg), m.p. 237–239°, $[\alpha]_D$ +36°, identical (mixed m.p. and IR spectrum) with samples prepared from cyclamigenin A[1] and C diacetates.

Cyclamiretin D diethyl acetal (XIV)

Cyclamiretin D (50 mg) in dry EtOH (25 ml) saturated with HCl was stored (2 days) at 0°, the soln evaporated to dryness at 0–5°, and the residue crystallized from MeOH to yield the *diethyl acetal* (15 mg), m.p. 340–343°, $[\alpha]_D$ +119°, ν_{max} 3410(s), 1105(s), 1087(s), 827(m) cm^{-1}. (Found: C, 72·8; H, 10·8. Calc. for $C_{34}H_{58}O_5 . MeOH$: C, 72·6; H, 10·8%). Acetylation of the alcohol, with Ac_2O in pyridine at room temp, furnished the corresponding *diacetate*, m.p. 318–320° (from hexane–EtOH), $[\alpha]_D$ +90°. (Found: C, 72·1; H, 9·8. Calc. for $C_{38}H_{62}O_7$: C, 72·3; H, 9·9%).

Cyclamiretin D (100 mg) in EtOH (50 ml) was refluxed (3 hr) with 10% HCl aq (50 ml). The product (97 mg), m.p. 239–241°. $[\alpha]_D$ +35°, precipitated by addition of water (100 ml), was identical (mixed m.p. and IR spectrum) with the starting material and thin-layer chromatography showed no trace of cyclamigenins A[1] or C.

Cyclamigenin A[2] (V)

The crude cyclamigenin B acetate (790 mg) from fraction C of the mixture of sapogenins[1] was dissolved in benzene and chromatographed on alumina (40 g). Elution with benzene (1·4 l.) gave a fraction (390 mg) crystallization of which from $CHCl_3$—MeOH furnished *cyclamigenin A*[2] *acetate* as fine needles, m.p. 231–233°, $[\alpha]_D$ −2°, ν_{max} 1735(s), 1705(s), 1245(s), 1023(s), 896(s) cm^{-1}, τ 9·14 (2Me), 9·09 (Me), 9·08 (d,

J = 7 Hz, Me), 8·76 (Me), 8·37 (Me), 7·96 (Ac), 7·25 (d, J = −15 Hz; H-15), 6·48 (d, J = −8 Hz, H-28), 6·08 (d, J = −8 Hz, H-28), 5·5 (m, H-3). (Found: C, 74·6; H, 10·2. Calc. for $C_{31}H_{48}O_4$. MeOH: C, 74·3; H, 10·1%). Mass spectrum (*m/e*)/% relative abundance 484 (M^+)/12, 483/9, 482/24, 315/37, 249/8, 234/27, 221/41, 219/42, 203/64, 191/49, 190/58, 189/100. Saponification of the acetate yielded *cyclamigenin A*[2] as feathery needles, m.p. 208–209° (from MeOH), $[\alpha]_D$ −17°, ν_{max} 3400(s), 1704(s), 1028(s), 890(s) cm^{-1}. (Found: C, 76·2; H, 10·5. Calc. for $C_{29}H_{46}O_3$. MeOH: C, 75·9; H, 10·6%).

Further elution of the column with benzene (1·5 l.) gave a mixture (380 mg) of cyclamigenin A[2] and B acetates.

Cyclamigenin D (VII)

(a) Cyclamigenin D acetate, isolated as described previously,[1] had m.p. 272–274°, $[\alpha]_D$ +3 ± 1°, ν_{max} 2825(m), 1735(s), 1707(s), 1143(s), 1105(s), 1071(s), 1002(s), 890(m) cm^{-1}, τ 9·14 (4Me), 8·98 (Me), 8·77 (Me), 7·99 (Ac), 7·3 (d, J = −16 Hz, H-15), 6·52 (2MeO), 6·56 (d, J = −8 Hz, H-28), 6·15 (d, J = −8 Hz, H-28), 5·76 (s, H-30), 5·5 (m, H-3). (Found: C, 73·5; H, 9·7. Calc. for $C_{34}H_{54}O_6$: C, 73·1; H, 9·7%). Mass spectrum (*m/e*)/% relative abundance 558 (M^+)/0·2, 526/0·5, 308/0·8, 249/0·3, 189/1, 75/100. Saponification of the acetate gave *cyclamigenin D* as needles, m.p. 325–327°, $[\alpha]_D$ −5°, ν_{max} 2825(m), 1707(s), 1143(s), 1105(s), 1003(s), 889(s) cm^{-1}. (Found: C, 74·4; H, 10·1. Calc. for $C_{32}H_{52}O_5$: C, 74·4; H, 10·1%). Mass spectrum (*m/e*)/% relative abundance 516 (M^+)/0·2, 526/0·5, 308/0·8, 249/0·3, 189/1, 75/100.

(b) A suspension of cyclamigenin B acetate (200 mg) in anhydrous MeOH (3 ml) was stirred (1 hr) at room temp with BF_3-etherate (0·5 ml), the mixture diluted with MeOH (5 ml), filtered, and the solid washed with cold MeOH (5 ml). Preparative TLC (alumina/benzene) afforded cyclamigenin B acetate (75 mg), m.p. 306–308°, $[\alpha]_D$ −9°, and VIII (12 mg), m.p. 271–273°, $[\alpha]_D$ +4°, identical (mixed m.p. and IR spectrum) with cyclamigenin D acetate. Alkaline hydrolysis of the acetate furnished the corresponding alcohol (VII), m.p. 324–326°, $[\alpha]_D$ −5°, identical (mixed m.p. and IR spectrum) with cyclamigenin D.

Acetolysis of cyclamigenin D

Cyclamigenin D (50 mg) in Ac_2O (3 ml) was heated (30 min) at 115–120° with *p*-toluenesulphonic acid (30 mg), the mixture poured into ice–water (25 ml), and worked up through ether. A soln of the product in AcOH (2 ml) was left (30 min) at room temp with CrO_3 (25 mg) in AcOH (2 ml), the mixture was diluted with water (10 ml), the precipitated solid washed with water, and crystallized from MeOH aq to yield needles (21 mg) of 3β,28-diacetoxy-16-keto-olean-12-en-30-oic acid, m.p. 275–277° (dec), $[\alpha]_D$ +11°, identical (mixed m.p. and IR spectrum) with a sample prepared[3] from cyclamiretin D diacetate.

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