Terpenoids. LVII.[1] Mass Spectral and Nuclear Magnetic Resonance Studies of Pentacyclic Triterpene Hydrocarbons[2]

JERRY KARLINER AND CARL DJERASSI

Department of Chemistry, Stanford University, Stanford, California

Received January 11, 1966

As an extension of earlier studies,[3] the mass spectra of Δ^{12}-oleanene, Δ^{12}-ursene, and deuterated analogs were determined. The use of deuterated derivatives served to confirm some fragmentation modes postulated earlier and caused revision of others. The intense and highly characteristic peak at *m/e* 203 was shown to be a composite fragment ion and its genesis is discussed. The synthesis of deuterated derivatives is described and suggested mechanisms for the formation of fragment ions are given. With the aid of high-resolution nmr spectroscopy and the use of deuterated derivatives, it was possible to identify all of the methyl signals in the nmr spectrum of Δ^{12}-oleanene.

A detailed study of the mass spectral fragmentation patterns of pentacyclic triterpenes by noting peak shifts in various derivatives has already been described.[3,4] This has led to important generalizations, particularly in Δ^{12} compounds, and has been extremely useful in structural elucidation studies of Δ^{12}-oleanene and Δ^{12}-ursene derivatives.[4,5]

As described earlier,[3] the dominant process upon electron impact of triterpenes containing a double bond at C-12 results from abstraction of an electron from the olefinic π bond, followed by a retro-Diels–Alder type[6] opening of ring C. The retro-Diels–Alder fragment then undergoes further decomposition reactions, and it is toward these subsequent fragmentations that the main emphasis of the present studies are directed. Precise location of a fragment ion can be determined by deuterium labeling as well as substituent labeling. However, it is only with the aid of isotope labeling that hydrogen-transfer reactions can be detected. Furthermore, the exact nature of the *m/e* 203 fragment ion (*vide infra*) necessitated investigating the mass spectra of deuterated derivatives. It is for these reasons that a program was initiated to prepare deuterium-labeled derivatives of Δ^{12}-oleanene and Δ^{12}-ursene. Since some of these compounds were to be deuterated at the tertiary methyl groups (of which there are eight), it was also decided to investigate the nuclear magnetic resonance spectra of Δ^{12}-oleanene and Δ^{12}-ursene.

Synthetic Studies

The synthetic transformations used for the preparation of deuterium-labeled compounds in the β-amyrin series are outlined in Chart I. Oleanolic acid (I) was oxidized by the Jones procedure[7] to oleanonic acid (II) and the latter was converted by a modified Huang-Minlon reaction[8] to oleanenic acid (III). Lithium aluminum hydride reduction of III afforded the primary alcohol IV, which was transformed to the aldehyde V by oxidation with pyridinium trifluoroacetate and dicyclohexylcarbodiimide in dimethyl sulfoxide according to the method of Pfitzner and Moffat.[9] Upon treatment with ethanedithiol, Δ^{12}-oleanen-28-al (V) afforded the dithioacetal VI, which led to the desired Δ^{12}-oleanene-28,28-d_2 (VII) upon desulfurization with deuterium-containing Raney nickel.[10]

Methyl acetylglycyrrhetate[11] (VIII) served as starting material for the preparation of the Δ^{12}-oleanene derivative isotopically labeled at C-30. Hydrogenation of VIII with platinum oxide in acetic acid furnished the 11-deoxy derivative IX,[12] which upon saponification afforded the hydroxy acid X. Chromic acid oxidation[7] followed by Huang-Minlon reduction[8] gave Δ^{12}-oleanene-30-carboxylic acid (XII). The same procedure (XII → XIII → XIV → XV → XVI) was employed for converting XII to the desired Δ^{12}-oleanene-30,30-d_2 (XVI) as has already been described above for similar transformations at C-28.

β-Amyrin (XVII) was used for the preparation of analogs of Δ^{12}-oleanene (XXI) deuterated in rings A and C. Jones oxidation[7] of XVII furnished Δ^{12}-oleanen-3-one (XVIII) and the latter was treated with *p*-toluenesulfonylhydrazide in methanol–dioxane to afford XIX. The tosylhydrazone was not isolated but was reduced directly to Δ^{12}-oleanene-3,3-d_2 (XX) by means of lithium aluminum deuteride, using deuterium oxide in the work-up procedure.[13] Δ^{12}-Oleanene (XXI) was prepared from XVIII by the Huang-Minlon procedure[8] and oxidized to the α,β-unsaturated ketone XXII by heating under reflux with chromium trioxide in acetic acid.[14] As shown previously,[12]

(1) For paper LVI, see O. Kennard, L. R. di Sanseverino, H. Vorbrüggen, and C. Djerassi, *Tetrahedron Letters*, 3433 (1965). The present article should also be considered part CV in the series, "Mass Spectrometry in Structural and Stereochemical Problems."

(2) Financial assistance (Grant No. GM-06840) from the National Institutes of Health of the U. S. Public Health Service is gratefully acknowledged.

(3) C. Djerassi, H. Budzikiewicz, and J. M. Wilson, *Tetrahedron Letters*, 263 (1962); H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).

(4) J. S. Shannon, *Australian J. Chem.*, **16**, 683 (1963).

(5) *Inter alia*, B. Tursch, *et al.*, *J. Org. Chem.*, **28**, 2390 (1963); S. Huneck and G. Snatzke, *Chem. Ber.*, **98**, 120 (1965); R. O. Donchaí and J. B. Thomson, *Tetrahedron Letters*, 2223 (1965); J. B. Thomson, *ibid.*, 2229 (1965); M. Alauddin, T. A. Bryce, E. Clayton, M. Martin-Smith and G. Subramanian, *J. Chem. Soc.*, 4611 (1965); L. H. A. Elgamal, M. B. E. Fayez, and G. Snatzke, *Tetrahedron*, **21**, 2109 (1965); I. P. Varshney, *et al.*, *Tetrahedron Letters*, 1187 (1965); R. Teschesche and G. Wulff, *ibid.*, 1569 (1965); S. Nakamura, *et al.*, *ibid.*, 2017 (1965); B. Tursch, J. Leclerq, and G. Chiurdoglu, *ibid.*, 4161 (1965); R. E. Taylor-Smith, *Tetrahedron*, **21**, 3721 (1965).

(6) For a discussion and other references of electron-impact induced retro-Diels–Alder reactions, see H. Budzikiewicz, J. I. Brauman, and C. Djerassi, *ibid.*, **21**, 1855 (1965).

(7) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(8) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2847 (1946).

(9) K. E. Pfitzner and J. G. Moffat, *ibid.*, **85**, 3027 (1963).

(10) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **85**, 2091 (1963).

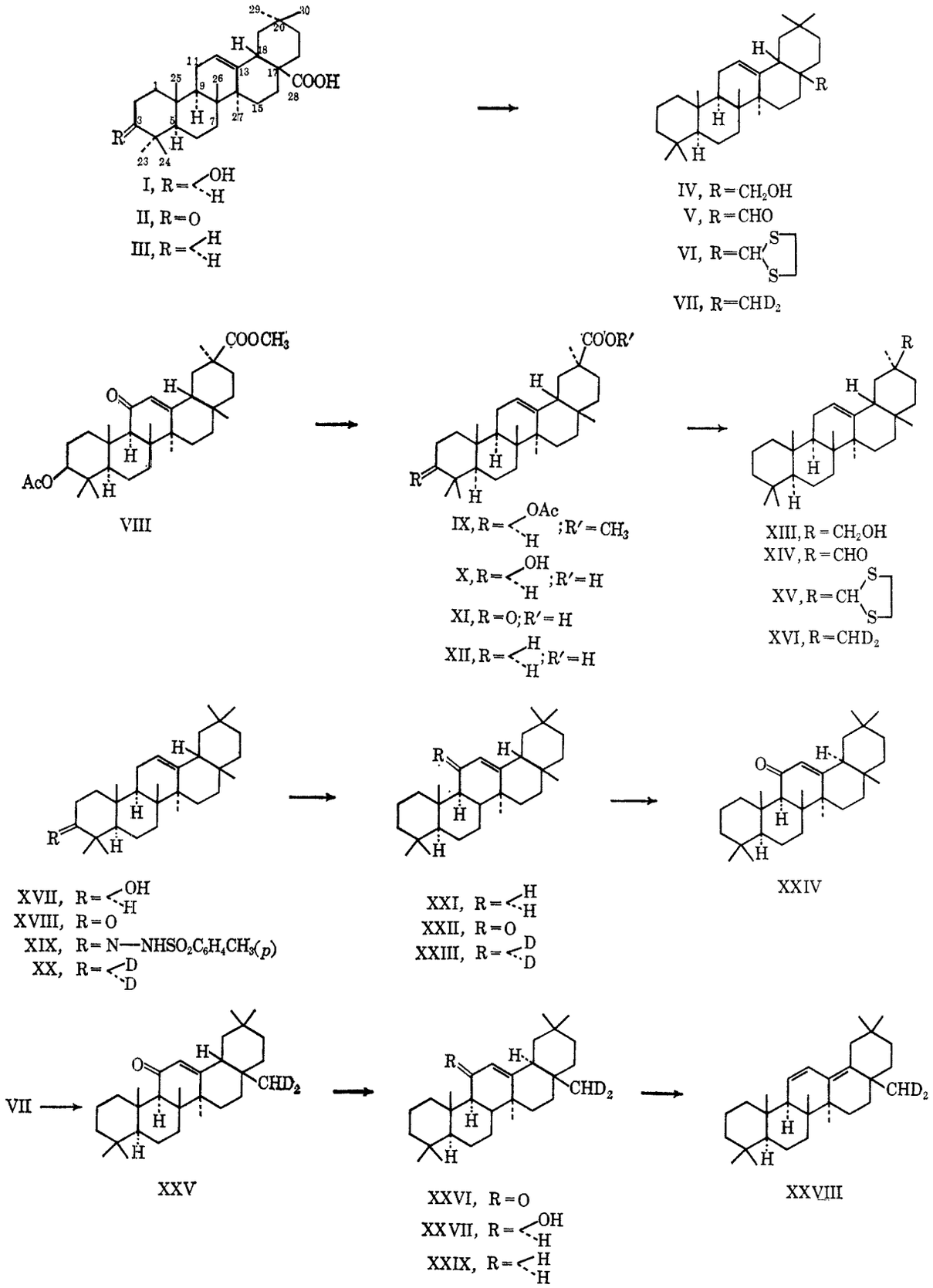
(11) L. Ruzicka and H. Leuenberger, *Helv. Chim. Acta*, **19**, 1402 (1936); L. Ruzicka, M. Furter, and H. Leuenberger, *ibid.*, **20**, 312 (1937); C. Djerassi and C. M. Foltz, *J. Am. Chem. Soc.*, **76**, 4085 (1954).

(12) L. Ruzicka, H. Leuenberger, and H. Schellenberg, *Helv. Chim. Acta*, **20**, 1271 (1937).

(13) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Chem. Ber.*, **98**, 3236 (1965).

(14) R. Budziarek, W. Manson, and F. S. Spring, *J. Chem. Soc.*, 3336 (1951).

CHART I



the oxygen function in Δ^{12}-oleanen-11-ones can be removed by hydrogenolysis with platinum oxide in acetic acid. This reaction provided a convenient method for introducing two deuterium atoms at C-11. Thus, treatment of a suspension of XXII and platinum oxide in O-deuterioacetic acid[15] with deuterium gas resulted in the formation of Δ^{12}-oleanene-11,11-d_2 (XXIII).

In an attempt to evaluate the role of stereochemical factors in the formation of the m/e 203 fragment (*vide*

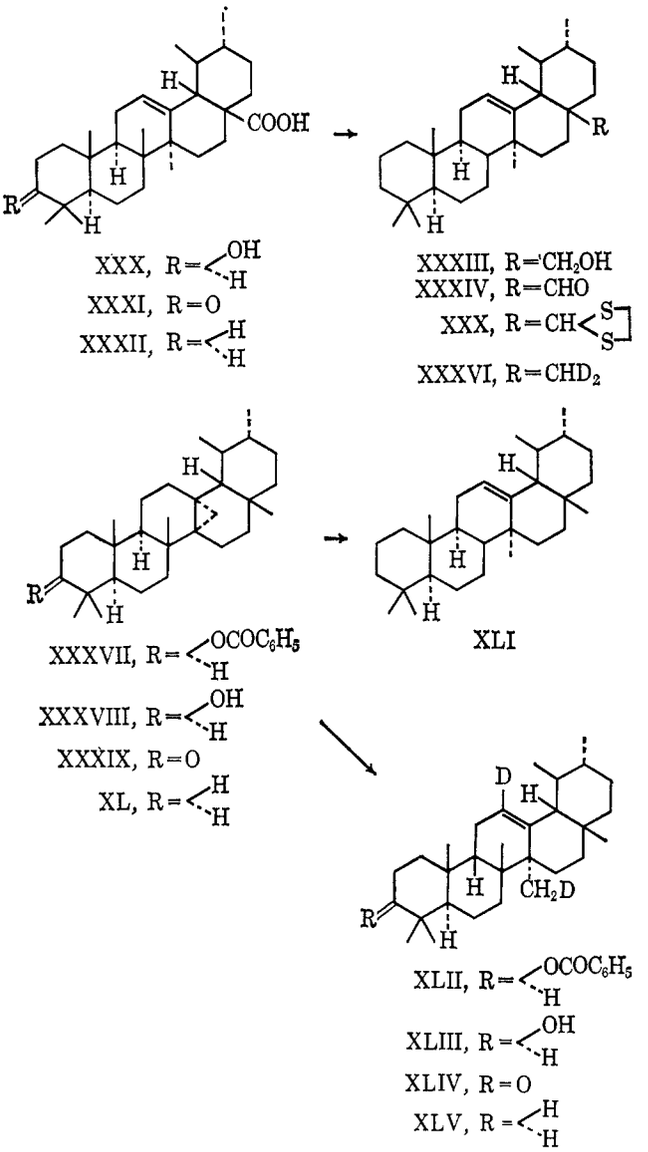
(15) M. J. S. Dewar and R. C. Fahey, *J. Am. Chem. Soc.*, **85**, 2245 (1963).

infra), it was necessary to prepare the 18α isomer of VII [*i.e.*, 18α-Δ^{12}-oleanene-28,28-d_2 (XXIX)]. The key step in its synthesis was the base-catalyzed inversion at C-18.[14] Prior to performing this transformation in the deuterated series, the reaction was attempted on the nondeuterated material XXII. Thus, treatment of XXII with boiling 15% potassium hydroxide in ethanol for 52 hr[14] resulted in the formation of an equilibrium mixture of XXII and XXIV,[16] which could be partially separated by column chromatography. The material eluted first was shown to be 18α-Δ^{12}-oleanen-11-one (XXIV) by comparing its ultraviolet spectrum and in particular its optical rotatory dispersion curve with the corresponding spectra of the 18β isomer XXII. Δ^{12}-Oleanen-11-one (XXII) exhibited λ_{max}^{EtOH} 250 mμ, while its 18α isomer XXIV exhibited λ_{max}^{EtOH} 244 mμ, consistent with a similar bathochromic shift noted previously upon changing the configuration at C-18 in a Δ^{12}-en-11-one.[14] Even more significant was a comparison of the optical rotatory dispersion curves of XXII and XXIV, reproduced in Figure 1. The change to negative rotation in XXIV is in complete accord with the earlier finding[17] that the rotatory dispersion curve of a Δ^{12}-11-keto chromophore is shifted to negative values upon isomerization of the D/E *cis* to the *trans* ring juncture.

After the completion of work with the model system, the synthesis of 18α-Δ^{12}-oleanene-28,28-d_2 (XXIX) was undertaken. Allylic oxidation of VII to XXV[14] followed by base equilibration as described above afforded 18α-Δ^{12}-oleanen-11-one-28,28-d_2 (XXVI). Attempted hydrogenolysis of the latter to the desired XXIX by employing Adams catalyst in acetic acid, according to the directions of Spring and co-workers,[14] failed and only starting material could be obtained, in spite of the success of this procedure in the 18β series (XXII → XXIII). As an alternate approach, XXVI was reduced with lithium aluminum hydride[18] to 18α-Δ^{12}-oleanen-11-ol-28,28-d_2 (XXVII) and the allylic alcohol was subjected to hydrogenolysis with platinum oxide, in a manner reported[18] to produce the 18α-Δ^{12} moiety. However, the only hydrocarbon obtained by this procedure was $\Delta^{11,13(18)}$-oleandiene-28,28-d_2 (XXVIII), characterized by its mass spectrometric molecular weight (M^+ ion at m/e 410) and the typical ultraviolet absorption[19] at 242, 250, and 259 mμ. The preparation of the desired dideuterated compound XXIX was finally achieved by treating the allylic alcohol XXVII with lithium metal in ethylamine.[20]

The synthesis of labeled α-amyrin derivatives is outlined in Chart II. Ursolic acid (XXX) was transformed to Δ^{12}-ursene-28,28-d_2 (XXXVI) in the same manner (XXX → XXXI → XXXII → XXXIII → XXXIV → XXXV → XXXVI) as discussed in the preparation of Δ^{12}-oleanene-28,28-d_2 (VII) from oleanolic acid (I).

CHART II



Phyllanthyl benzoate[21,22] (XXXVII) was converted to Δ^{12}-ursene (XLI) according to literature directions[21] (XXXVII → XXXVIII → XXXIX → XL → XLI). Treating XXXVII with anhydrous deuterium chloride in anhydrous alcohol-free chloroform resulted in the formation of α-amyrin benzoate XLII deuterated at C-27. As reported by Barton and de Mayo[21] the cyclopropane-opening reaction afforded a considerable amount of overdeuterated product (20% d_0, 35% d_1, 42% d_2, 3% d_3). An examination of the integrated areas corresponding to the protons at C-3 (centered at δ 4.74) and C-12 (centered at δ 5.16) in the nuclear magnetic resonance spectrum of XLII indicated the presence of 18% of deuterium at C-12.[23] The predominantly dideuterio-α-amyrin benzoate XLII was then converted to XLV in three steps by lithium aluminum hydride reduction to XLIII, followed by chromic acid oxidation[7] to XLIV and subsequent Huang-Minlon reduction[8] to XLV.

(16) This is in contrast to a report in the literature[14] that base-catalyzed equilibration of an 18β-Δ^{12}-11-one results in complete conversion to the corresponding 18α isomer.

(17) C. Djerassi, J. Osiecki, and W. Closson, *J. Am. Chem. Soc.*, **81**, 4587 (1959).

(18) G. G. Allen and F. S. Spring, *J. Chem. Soc.*, 2125 (1955).

(19) P. Boiteau, B. Pasich, and R. Ratsimamonga, "Les Triterpenoids en Physiologie Végetale et Animale," Gauthier-Villars, Ed., Paris, 1964, p 589.

(20) See A. S. H. Hallsworth, H. B. Henbest, and T. I. Wrigley, *J. Chem. Soc.*, 1969 (1957).

(21) D. H. R. Barton and P. de Mayo, *ibid.*, 2178 (1953).

(22) We gratefully acknowledge a generous gift of this material from Professor Barton (Imperial College of Science and Technology, London).

(23) Barton and de Mayo also assigned C-12 as the site of excess deuterium incorporation.

TABLE I[a]

PRINCIPAL MASS SPECTRAL PEAKS OF Δ^{12}-OLEANENE, Δ^{12}-URSENE, AND DEUTERATED ANALOGS

Compd	Isotopic purity, %	m/e M+	M − 153	M − 192	M − 207	M − 219	M − 221
Δ^{12}-Oleanene (XXI)		410	257	218	203	191	189
Δ^{12}-Oleanene-28,28-d_2 (VII)	7 d_0	412	259 (85)		205 (65)	193 (10)	191 (70)
	24 d_1		257 (15)	220 (q)	203 (35)	191 (90)	189 (30)
	63 d_2						
	6 d_3						
Δ^{12}-Oleanene-30,30-d_2 (XVI)	7 d_0	412	259 (85)	220 (q)	205 (65)	193 (20)	191 (75)
	19 d_1		257 (15)		203 (35)	191 (80)	189 (25)
	62 d_2						
	12 d_3						
Δ^{12}-Oleanene-11,11-d_2 (XXIII)	3 d_0	412	259 (q)	*b*	205 (q)	191 (q)	191 (10)
	17 d_1						189 (90)
	80 d_2						
Δ^{12}-Oleanene-3,3-d_2 (XX)	9 d_0	412	259 (5)	218 (q)	203 (q)	193 (95)	191 (10)
	42 d_1		257 (95)			191 (5)	189 (90)
	49 d_2						
18α-Δ^{12}-Oleanene-28,28-d_2 (XXIX)	17 d_0	412	259 (85)	220 (q)	205 (65)	193 (20)	191 (80)
	28 d_1		257 (15)		203 (35)	191 (80)	189 (20)
	50 d_2						
	5 d_3						
Δ^{12}-Ursene (XLI)		410	257	218	203	191	189
Δ^{12}-Ursene-28,28-d_2 (XXXVI)	7 d_0	412	259 (85)	220 (q)	205 (65)	193 (15)	191 (70)
	23 d_1		257 (15)		203 (35)	191 (85)	189 (30)
	62 d_2						
	8 d_3						
Δ^{12}-Ursene-12,27-d_2 (XLV)[c]	20 d_0	412	259 (80)	220 (q)	205 (q)	191 (q)	191 (20)
	35 d_1		258 (20)				190 (80)
	42 d_2						
	3 d_3						

[a] Indicates the calculated per cent shift of the peak in compounds specifically labeled with deuterium. (A sample calculation is presented in the Experimental Section.) The symbol (q) refers to a quantitative shift (*i.e.*, >95%). [b] Per cent isotopic purity determined from this peak in compound XXIII. [c] It was assumed that the d_1 species consisted of Δ^{12}-ursene-27-d_1.

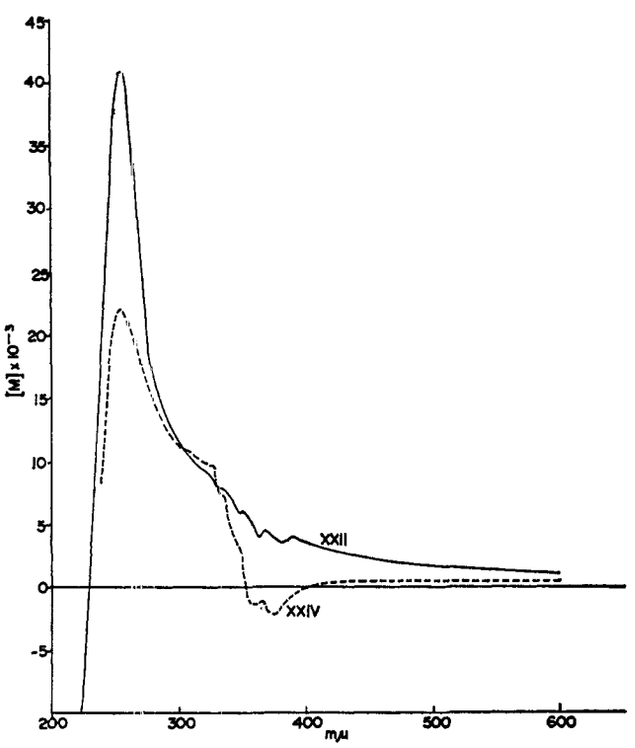


Figure 1.—Optical rotatory dispersion spectra of Δ^{12}-oleanen-11-one (XXII) and 18α-Δ^{12}-oleanen-11-one (XXIV).

Discussion of Mass Spectra

The mass spectrum of Δ^{12}-oleanene (XXI) is reproduced in Figure 2, while that of Δ^{12}-ursene (XLI) is shown in Figure 3. It can be seen that the two spectra are very similar, giving rise to fragments at identical *m/e* values. However, one important difference is to be noted. In the mass spectrum (Figure 2) of Δ^{12}-oleanene, the fragment ion at *m/e* 203 is more intense than the peak at *m/e* 191, while the reverse is true (*m/e* 191 more intense than *m/e* 203) in the mass spectrum of Δ^{12}-ursene (Figure 3). Although this observation can be used to differentiate Δ^{12}-oleanene (XXI) from Δ^{12}-ursene (XLI), using such a criterion to assign the skeletal structure of an unknown triterpene should not be attempted until a detailed study comparing the mass spectral characteristics of identical Δ^{12}-oleanene and Δ^{12}-ursene derivatives is performed.

The calculated peak shifts of the various deuterated derivatives, after correcting for isotopic impurties, are shown in Table I. An example of such a calculation is described in the Experimental Section. Inherent in the calculated peak shifts of 18α-Δ^{12}-oleanene-28,28-d_2 (XXIX) is the assumption that Δ^{12}-oleanene (XXI) and 18α-Δ^{12}-oleanene exhibit similar fragments in the high mass region (*i.e.*, *m/e* >185). Since the mass spectra of Δ^{12}-oleanene (Figure 2) and Δ^{12}-ursene (Figure 3) are qualitatively the same, the peak migrations and mechanistic genesis of fragment ions other than *m/e* 203 of these two classes will be considered together. The individual peaks will now be discussed in detail.

Peak M − 153 (*m/e* 257 in Figures 2 and 3).—This peak represents the most abundant fragment ion above *m/e* 218. Inspection of Table I reveals that a major part of this peak is shifted to *m/e* 259 in compounds VII, XVI, and XXXVI, and is displaced entirely to *m/e* 259 in the 11,11-dideuterated substance XXIII. This

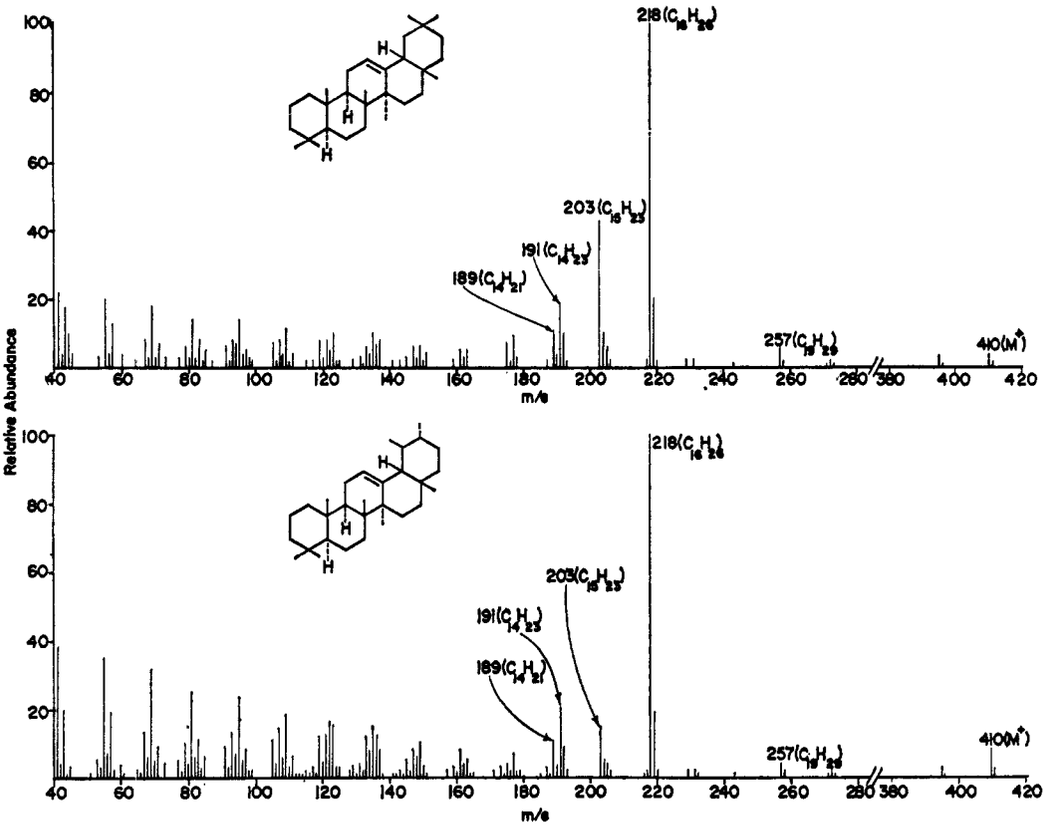
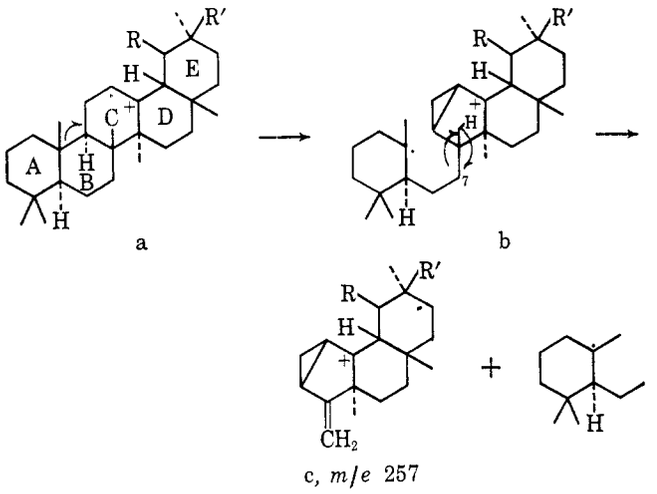


Figure 2.—Mass spectrum of Δ^{12}-oleanene (XXI).
Figure 3.—Mass spectrum of Δ^{12}-ursene (XLI).

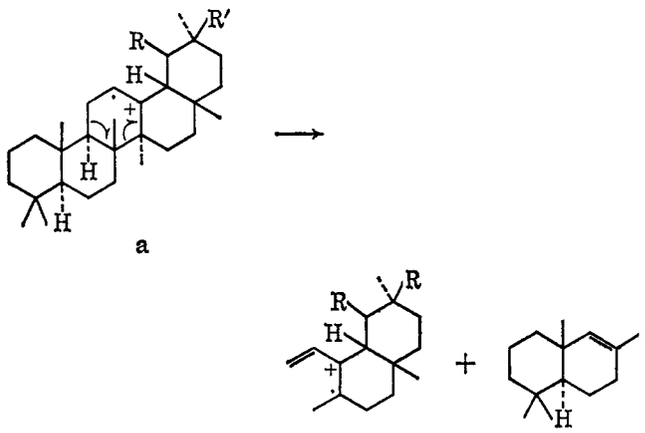


R = CH_3; R′ = H or R = H; R′ = CH_3

implicates rings C, D, and E for the charge-containing portion, consistent with the observation that the mass spectrum of the 3,3-d_2 analog XX exhibits this peak at *m/e* 257. Furthermore, the mass spectrum of XLV demonstrates that C-27 is also retained in this fragment. A mechanism consistent with these data involves homolytic cleavage[24] of the 9–10 bond in the molecular ion a[3,4] to afford b, followed by hydrogen transfer from C-26 to C-7 with concomitant homolysis of the 7–8 bond to afford the resonance stabilized species c (*m/e* 257) above.

(24) A fishhook (↶) is used to designate the shift of a single electron, while the movement of an electron pair is signified by an arrow (↷): H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco Calif., 1964, pp 1–3.

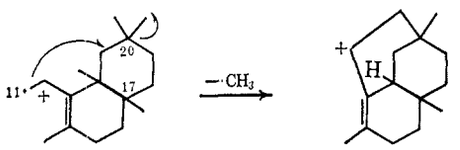
Peak M − 192 (*m/e* **218 in Figures 2 and 3**).—The intense and diagnostically important *m/e* peak (base peak in XXI and XLI) has been discussed in considerable detail elsewhere.[3,4,6] It is sufficient to note here that the earlier mechanistic interpretation for the formation of the *m/e* 218 species by a retro-Diels–Alder decomposition (*i.e.*, a → d)[3,4,6] is consistent with the spectra of all the deuterated derivatives examined in this study. For the sake of brevity only one of the possible resonance forms of d is shown.



R = CH_3; R′ = H or R = H; R′ = CH_3

Peak M − 207 (*m/e* **203 in Figures 2 and 3**).—The fragment ion appearing at *m/e* 203 in the mass spectra of Δ^{12}-oleanene (XXI) and Δ^{12}-ursene (XLI) proved to be the most interesting peak from a mechanistic standpoint and in fact was the chief reason why the present deuterium-labeling studies were undertaken. This

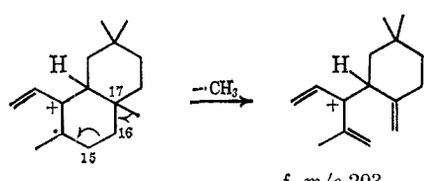
moiety results from further loss of 15 mass units from the retro-Diels–Alder fragment d. The mass spectrum of Δ^{12}-oleanene-28,28-d_2 (VII) was investigated in order to determine whether the C-17 substituent was involved in the *m/e* 218 → *m/e* 203 fragmentation and it was found that VII accounted for only *ca.* one-third loss of the C-28 methyl group. Similarly the mass spectrum of Δ^{12}-oleanene-30,30-d_2 (XVI) exhibited *ca.* one-third loss of the axial C-20 substituent and, therefore, it is reasonable to assume that the remaining one-third loss of 15 mass units results from expulsion of the C-29 methyl group. It was thought that perhaps loss of the methyl groups from C-20 was facilitated by a ring-closure reaction, as shown in d → e. An



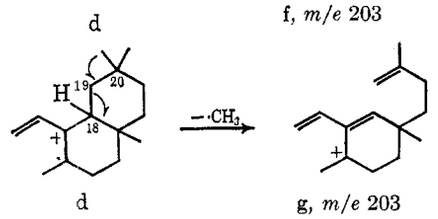
d e, *m/e* 203

examination of Dreiding models indicates that such ring formation is feasible. However, the molecular models suggest that in the 18α series, bond formation between C-11 and C-20 is not possible. Therefore, in order to determine whether ion *e* was an important contributor to the *m/e* 203 fragment, 18α-Δ^{12}-oleanene-28,28-d_2 (XXIX) was prepared and its mass spectrum determined. If path d → e were operating in the electron impact induced decomposition of Δ^{12}-oleanene, then, since such a pathway is not possible in the D/E *trans*-fused compound XXIX, the latter would be expected to lose the C-28 methyl group to a greater extent than observed for Δ^{12}-oleanene-28,28-d_2 (VII). However, it was found that in the mass spectrum of XXIX expulsion of the C-28 methyl was responsible for 35% of the *m/e* 218 → *m/e* 203 fragmentation. This figure coincides with that noted in the loss of the C-28 methyl grouping in VII and forces one to conclude that d → e does not represent a significant pathway for the genesis of the *m/e* 203 species.

The finding that the *m/e* 203 results from the retro-Diels–Alder fragment d by equal loss of the methyl substituents at C-17 or C-20 can be explained in the following manner. Fragment d may suffer a loss of the C-28 methyl group, while undergoing homolytic scission of the 15–16 bond to afford the resonance stabilized dienyl cation f. Alternatively, d, upon expulsion of a methyl group bonded to C-20 (either the C-29 or the C-30 methyl group) with concomitant homolytic cleavage of the 18–19 bond results in another



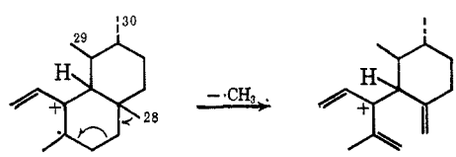
d f, *m/e* 203



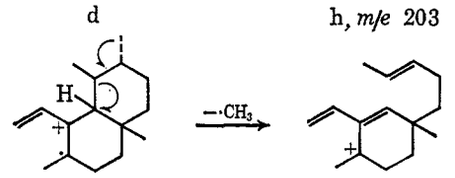
d g, *m/e* 203

dienyl cation g. In Δ^{12}-oleanene and Δ^{12}-ursene derivatives, oxygen-containing substituents at C-17 or C-20 ($COOCH_3$, CH_2OH, CH_2OAc, or CHO) are lost in preference to methyl from the retro-Diels–Alder fragment ion owing to stabilization of the expelled radical by oxygen.

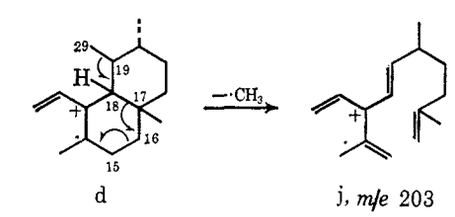
The mechanistic interpretation of the origin of the *m/e* 203 species in the oleanene series would lead one to predict, *a priori*, that in the ursene series the formation of the *m/e* 203 moiety would result from 50% loss of the C-28 methyl radical and 50% expulsion of the C-30 methyl radical, since Δ^{12}-ursene contains only a single methyl group at C-20. An examination of the mass spectrum of Δ^{12}-ursene-28,28-d_2 (see Table I) shows that, in fact, cleavage of the C-28 methyl group accounts for only 35% of the formation of the *m/e* 203 peak. It, therefore, appears that in Δ^{12}-ursene, as had been noted in Δ^{12}-oleanene, the loss of 15 mass units, upon generation of the *m/e* 203 fragment from the retro-Diels–Alder species d, involves all three ring-E methyl groups to the same extent. These considerations lead to three alternative fragmentation modes for the formation of the *m/e* 203 cleavage product. Paths d → h and d → i are analogous to those discussed for the similar decomposition of Δ^{12}-oleanene (see f and g) while route d → j involves expulsion of the C-29 methyl radical and homolytic fissiion of the 17–18 and 15–16 bonds.



d h, *m/e* 203

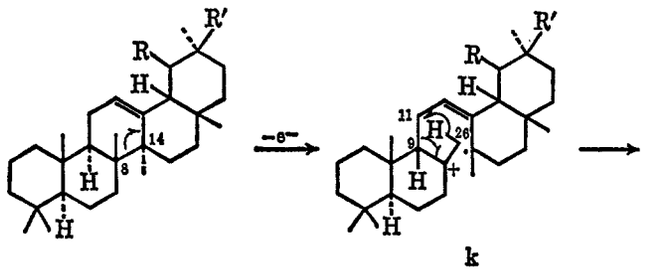


d i, *m/e* 203

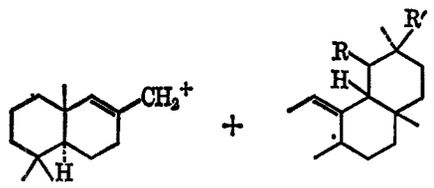


d j, *m/e* 203

Peak M − 219 (*m/e* 191 in Figures 2 and 3).—The results shown in Table I are in full agreement with previous studies on the origin of this peak.[3] Thus compound XX implicates ring A as being part of this fragment, while VII, XVI, and XXXVI indicate that a major portion of this peak does not involve rings D and E. Furthermore, the spectrum of XXIII reveals the absence of C-11 as part of the *m/e* 191 fragment and XLV shows that C-27 is likewise not involved. The mechanism previously proposed[3] for this species involved heterolytic fission of the allylically activated 8–14 bond to form k, consisting of a tertiary carbonium ion and an allylic radical. Hydrogen transfer from C-26 to C-11 with simultaneous bond breaking at 9–11 would then result in the formation of l, *m/e* 191. Although the results described in the present studies can

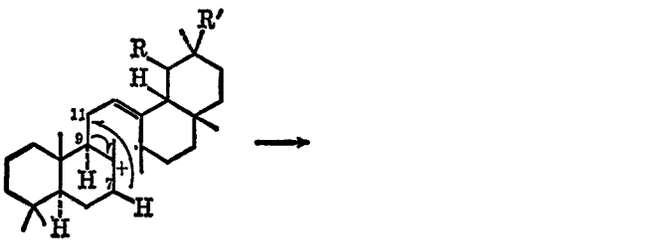


k

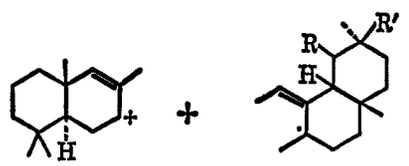


l, *m/e* 191

be reconciled with such a mechanism, hydrogen transfer from C-7 would be preferred to a similar transfer from C-26 (transfer of a secondary *vs.* a primary hydrogen atom)[25] and it appears that k → m is energetically more favorable than k → l as a representation of the generation of the *m/e* 191 fragment.



k

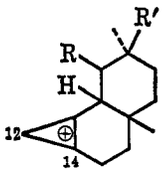


m, *m/e* 191

R=CH_3; R′=H or R=H; R′=CH_3

Peak M − 221 (*m/e* 189 in Figures 2 and 3).—The fragment occurring at *m/e* 189 has been found to be more complicated than thought earlier.[3] An examination of Table I reveals that the predominant composition of this peak involves the right-hand portion of the triterpene molecule. Thus, compounds VII and XXXVI indicate that C-28 is retained in this species, while compound XVI implicates C-30 as part of the fragment ion. A major part of the peak remains at *m/e* 189 in XX and XXIII demonstrating that ring A and C-11 are not involved in the *m/e* 189 species. A striking entry in Table I is that of compound XLV. The pronounced shift to *m/e* 190 in this compound indicates that the C-27 methyl group is lost. (The alternative explanation that a hydrogen atom from the vinylic C-12 position is expelled is unlikely.) These results lead to structure n, a stable cyclopropenium cation, as a representation of the *m/e* 189 fragment, although its manner of formation is not obvious.

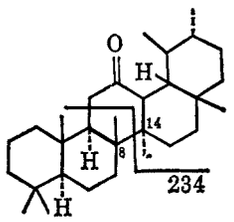
(25) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day Inc., San Francisco, Calif., 1964, p 11.



n, *m/e* 189

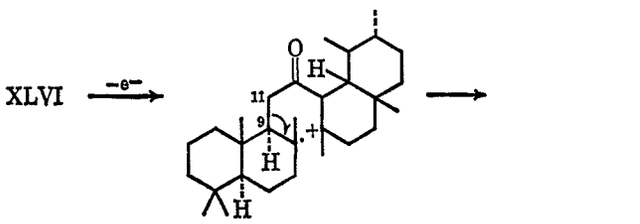
R=CH_3; R′=H or R=H; R′=CH_3

It seems appropriate at this point to suggest a mechanistic modification in the characteristic mass spectral cleavage reaction of 12-oxygenated triteroenes. Recently a comparison has been made[26] of $\Delta^{9(11)}$-12-ketones in steroids and triterpenes and this does not need to be considered further. However, saturated 12-ketones in triterpenes, such as 3β-acetoxy-12-oxoursane (XLVI) exhibit a base peak at *m/e* 234 which

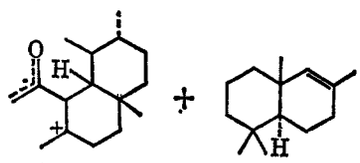


XLVI

was shown to be the fragment indicated schematically in formula XLVI. The formation of this *m/e* 234 fragment may be considered as occurring by a decomposition mode which is directly analogous to the generation of the base peak of 7-keto steroids.[27] Thus, cleavage of the 8-14 bond with expulsion of an electron results in the molecular ion o, which may then undergo homolysis of the 9–11 bond to furnish the resonance stabilized radical ion p (*m/e* 234).



o



p, *m/e* 234

Discussion of Nuclear Magnetic Resonance Spectra

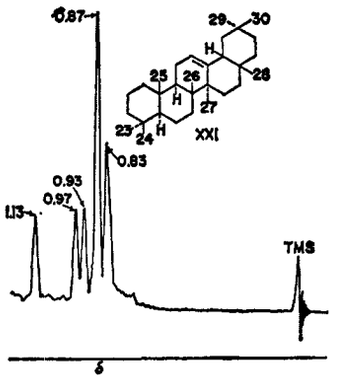
An early investigation of the nuclear magnetic resonance spectra of Δ^{12}-oleanene and Δ^{12}-ursene derivatives at 40 Mc revealed overlapping signals in the methyl region and specific assignments to most of the methyl groups could not be made.[28] Other reports involving nuclear magnetic resonance studies of pentacyclic triterpenes have been confined to lupane,[29]

(26) L. Tökés and C. Djerassi, *Steroids*, **6**, 493 (1965).

(27) R. Beugelmans, R. H. Shapiro, L. J. Durham, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2832 (1964).

(28) M. Shamma, R. E. Glick, and E. Glotter, *J. Org. Chem.*, **27**, 4512 (1962).

(29) J. M. Lehn and G. Ourisson, *Bull. Soc. Chim., France*, 1137 (1962).



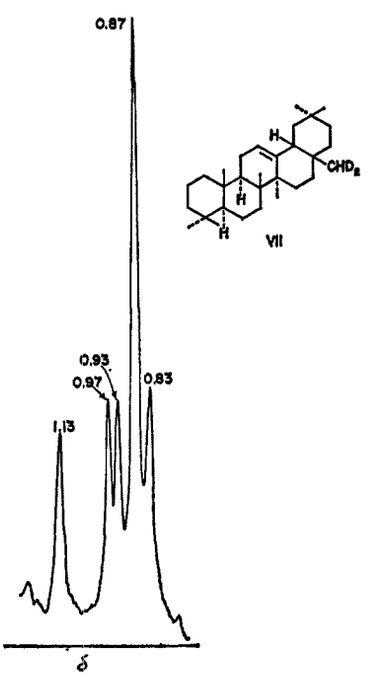
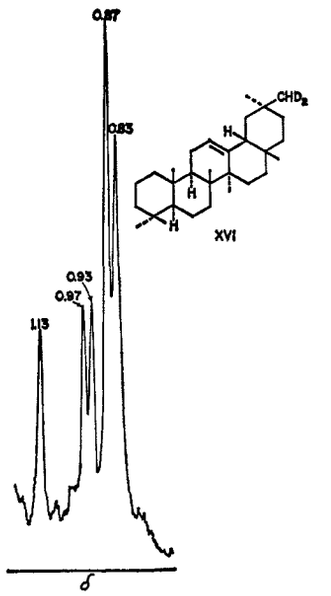


Figure 4.—Partial 100-Mc nuclear magnetic resonance spectrum (high-field region) of Δ^{12}-oleanene (XXI).

Figure 5.—Partial 100-Mc nuclear magnetic resonance spectrum (high-field region) of Δ^{12}-oleanene-28,28-d_2 (VII).



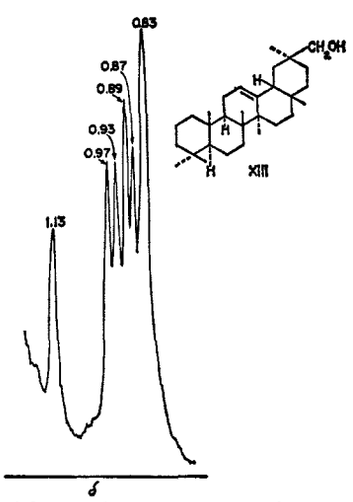


Figure 6.—Partial 100-Mc nuclear magnetic resonance spectrum (high-field region) of Δ^{12}-oleanene-30,30-d_2 (XVI).

Figure 7.—Partial 100-Mc nuclear magnetic resonance spectrum (high-field region) of Δ^{12}-oleanen-30-ol (XIII).

hopane,[30] betulin,[31] and Δ^{12}-oleanene[32] derivatives. The investigations involved determining the spectra of compounds differing in the nature or placement of a functional group and examining the shift of methyl peaks in such derivatives.

In view of the availability of deuteriated derivatives of the Δ^{12}-oleanene and Δ^{12}-ursene hydrocarbons, we decided to investigate the nmr spectra of these compounds. The nmr spectrum of Δ^{12}-ursene (XLI) was quite complex (even at 100 Mc) owing to second-order perturbation effects[33] exhibited by the two secondary methyl groups and was not examined further. However, the methyl region (δ 0.0–1.2) in the nmr spectrum of Δ^{12}-oleanene (XXI) was readily discernible and forms the basis of the present discussion.

The high-field region of the 100-Mc nmr spectra of Δ^{12}-oleanene (XXI), Δ^{12}-oleanene-28,28-d_2 (VII), Δ^{12}-oleanene-30,30-d_2 (XVI), and Δ^{12}-oleanen-30-ol (XIII) are shown in Figures 4–7, respectively. The parent substance XXI exhibits peaks corresponding to one methyl group at δ 1.13, 0.97, and 0.93, a signal at δ 0.87 due to three methyl groups, and a peak at δ 0.83 corresponding to two methyl groups. The spectrum of VII shows a marked decrease in the resonance at δ 0.83, thereby ascribing one of the methyl groups at this frequency to C-28 in XXI. Similarly, the area at δ 0.87 in Figure 4 diminishes in the spectrum of Δ^{12}-oleanene-30,30-d_2 (XVI), demonstrating that the C-30 methyl signal in Δ^{12}-oleanene (XXI) appears at δ 0.87. That the C-29 methyl resonance also occurs at δ 0.87 in the nmr spectrum of XXI can be seen by comparing the spectra of Δ^{12}-oleanene (Figure 4) with that of its 30-hydroxy derivative (XIII) (Figure 7). Of the three methyl resonances present at δ 0.87 in the parent hydrocarbon XXI, two methyl signals are deleted from this value and a new methyl peak appears at δ 0.89 in the spectrum of XIII. One of the methyl signals removed is obviously the one ascribed to C-30 (no such methyl group is present in XIII), while the C-29 methyl group has shifted to δ 0.89 owing to the

(30) S. Huneck and J. M. Lehn, *Bull. Soc. Chim., France*, 1702 (1963).

(31) J. M. Lehn and A. Vystrčil, *Tetrahedron*, **19**, 1733 (1963).

(32) B. Tursch, R. Savoir, and G. Chiurdoglu, *Bull. Soc. Chim. Belges* **75**, 107 (1966). We wish to thank Dr. Tursch for supplying us with a preprint of this paper.

(33) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 35.

small deshielding effect of the γ-hydroxyl group.[34] The remaining methyl resonance at δ 0.87 in Figure 4 is attributed to the C-23 methyl group since the latter has an environment similar to the methyl group at C-29 and is not associated with any unusual interactions which may give rise to shielding or deshielding phenomena.

The remaining methyl groups are involved in 1,3-diaxial interactions and will be considered together. It has previously been shown that axial methyl groups, upon introduction of an axial β-methyl group are deshielded by 0.05–0.10 ppm.[35] The methyl signal at lowest field (δ 1.13) in Figure 4 is undoubtedly due to the methyl protons at C-27. This observed deshielding is a consequence of the C-27 methyl group being homoallylic and suffering a 1,3-diaxial interaction with the 18–19 bond. Of the methyl groups which have not been accounted for, the C-24 and C-26 suffer one diaxial interaction (with C-25) while the C-25 methyl group is associated with two diaxial interactions (C-24 and C-26). This leads to the conclusion that the C-25 methyl group occurs at higher frequency than the other two methyl substituents and is accordingly ascribed to the resonance at δ 0.97. These considerations would also predict that the signals due to the C-24 and C-26 methyl protons would occur at the same frequency, as is obviously not the case. This apparent discrepancy can be explained upon examination of a Dreiding model of the Δ^{12}-oleanene molecule. The nonrigidity of ring C permits distortion of this molecule to occur. If distortion were to occur, in order to alleviate any of the 1,3-diaxial interactions present in XXI (C-24 *vs.* C-25; C-25 *vs.* C-26; C-27 *vs.* C-19), the C-26 methyl group would be forced into the region above the plane of the olefinic bond, a situation known to give rise to shielding effects.[36] On this basis, one of the methyl groups responsible for the peak at 0.83 is assigned to C-26, while the resonance at δ 0.93 is ascribed to the C-24 methyl group.

To summarize, the assignments of methyl groups in the high-field region in the nmr spectrum of Δ^{12}-oleanene (XXI) are

δ value	1.13	0.97	0.93	0.87	0.83
methyl group	27	25	24	23, 29, 30	26, 28

Experimental Section[37]

Method of Calculating Peak Shifts.—The generally low isotopic purity of products obtained by deuterium-containing Raney nickel desulfurization[10] necessitated the judicious calculation of peak shifts. An example of such a calculation for the *m/e* 203 fragment in Δ^{12}-oleanene-30,30-d_2 (XVI) is presented in Table II. Compound XVI contained 7% d_0 (*m/e* 410), 19% d_1 (*m/e* 411), 62% d_2 (*m/e* 412), and 12% d_3 (*m/e* 413).

Analysis of the mass spectrum of the unlabeled compound XXI indicated the relative abundance of fragments shown in Table II for the *m/e* 201–*m/e* 208 region, after correction for C^{13} content. The anticipated spectrum of XVI in this region, assuming complete retention of the isotope label, was then calculated by the following procedure. Of the total 7% d_0 species, 6% (79 × 7%, to the nearest integer) would be expected to appear at *m/e* 203, while 1% (10 × 7%) would result at *m/e* 205. Similarly, contributions of 15% (79 × 19%), 1% (5 × 19%), and 2% (10 × 19%) at *m/e* 204, 205 and 206, respectively, would be expected from the d_1 species. Performing the calculations for the 62% d_2 and 12% d_3 species and addition of the contributions to a given *m/e* value results in the predicted spectrum. Thus, if no loss of the C-30 methyl group had occurred, 51% of the peak group discussed should occur at *m/e* 205 and 7% at *m/e* 203; *i.e.*, 88% (51/58 × 100) of the total intensity of these two peaks should appear at *m/e* 205 and 12% (7/58 × 100) at *m/e* 203. Of the total intensity at *m/e* 205 and *m/e* 203 in the observed spectrum, 50.2% (88% × 57) would be expected at *m/e* 205 and 6.8% (12% × 57) at *m/e* 203 if the C-30 methyl group were not expelled. The deviation from these values indicate that 35% (50.2 − 33/50.2) × 100 = 33.5% to the nearest 5%) of the C-30 methyl group is lost in compound XVI.

TABLE II

ION CURRENT (%) OF *m/e* 201 TO *m/e* 208 REGION

	m/e							
Compd	201	202	203	204	205	206	207	208
Δ^{12}-Oleanene (XXI)	2	2	79	5	10	2	...	...
Δ^{12}-Oleanene-30,30-d_2 (XVI)	...	...	6	...	1	...	...	...
	...	...	...	15	1	2	...	...
	...	...	1	1	49	3	6	1
	...	...	...	...	...	9	1	1
Predicted	...	...	7	16	51	14	7	2
Obsd	3	3	24	10	33	12	11	4

Δ^{12}-Oleanene-28-carboxylic Acid (III).—Jones reagent[7] was added dropwise to a stirred solution of 690 mg of crude oleanolic acid (I) in acetone until an orange color persisted for 1 min. The mixture was then concentrated to one-half its volume, diluted with water, and extracted twice with ether. The combined ether extracts were washed twice with water and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure gave an oil which crystallized upon addition of a few drops of methanol. Recrystallization from methanol afforded a material (II, 440 mg) melting at 154–158°, which was not purified further but subjected directly to a modified Huang-Minlon reduction.[8]

The oxidation product II and 5 ml of 95% hydrazine in 20 ml of diethylene glycol and 15 ml of absolute ethanol were heated under reflux for 1 hr under nitrogen. After adding 2.1 g of potassium hydroxide pellets and heating under reflux for an additional 0.5 hr, the condenser was removed and the reaction mixture was warmed slowly until an internal temperature of 195° was attained. The condenser was returned and the reaction mixture was maintained at 195–205° for 4 hr while being kept under an atmosphere of nitrogen. After cooling to room temperature, the solution was poured into dilute hydrochloric acid, extracted with ether, and the ethereal extract was washed twice with water. Drying over anhydrous magnesium sulfate, followed by removal of solvent under diminished pressure, afforded 420 mg of product (III): mp 264–266° after recrystallization from methanol, $[\alpha]^{26.5}D$ +45.56 (*c* 0.72). *Anal.* Calcd for $C_{30}H_{48}O_2$: mol wt, 440. Found by mass spectrometry: mol wt, 440.

Δ^{12}-Oleanen-28-ol (IV).—To a stirred suspension of 110 mg of lithium aluminum hydride in anhydrous ether was added drop-

(34) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Ltd., London, 1959, p 53.

(35) G. Slomp, Jr., and B. R. McGarvey, *J. Am. Chem. Soc.*, **81**, 2200 (1959).

(36) See ref 34, p 129.

(37) The mass spectra were determined with a Consolidated Electrodynamics Corporation mass spectrometer, No. 21-103C, equipped with a direct inlet system. The ionizing current was kept at 50 μa, while the ionizing energy was maintained at 70 ev and the source temperature at 250°. The optical rotatory dispersion measurements were made on a Japan Spectroscopic Co. (Jasco) automatically recording spectropolarimeter Model ORD-5. Melting points were determined on a Kofler hot stage and are uncorrected. Analytical thin layer chromatoplates with a thickness of 0.25 mm of silica gel G (E. Merck A.G., Darmstadt) were used and the spots were detected by spraying with a 2% ceric sulfate solution in 2 *N* sulfuric acid. Preparative thin layer chromatoplates of 0.50-mm thickness were prepared from a slurry of silica gel H (E. Merck A. G., Darmstadt) in 5% silver nitrate solution and compounds were detected by viewing in a direct path of light. Nuclear magnetic resonance spectra were determined with Varian Associates Model A-60 and HR-100 nmr spectrometers using tetramethylsilane as an internal reference standard. We wish to express our appreciation to Mr. J. Smith for mass spectral determinations, to Dr. L. J. Durham for obtaining the nmr spectra, and to Messrs. E. Meier and J. Consul of the Stanford University Microanalytical Laboratory for microanalysis determinations.

wise a solution of 205 mg of Δ^{12}-oleanenic acid (III) in anhydrous ether and the resulting suspension maintained at reflux overnight while under nitrogen. While cooling in an ice–water bath, water was added dropwise to decompose excess hydride reagent. Dilute hydrochloric acid was added to dissolve the inorganic hydroxides and the two-phase system was separated. The ether layer was washed with water, 5% sodium bicarbonate, and water, and dried over anhydrous magnesium sulfate. Removal of solvent afforded Δ-^{12}-oleanen-28-ol (190 mg) which upon rcerystalline from chloroform–methanol furnished a microcrystalline material: mp 92–96°, $[\alpha]^{26.8}D$ +79.68° (*c* 0.74). *Anal.* Calcd for $C_{30}H_{50}O$: mol wt, 426. Found by mass spectrometry: mol wt, 426.

Δ^{12}-Oleanen-28-al (V).—A mixture of 55 mg of IV, 12 mg of pyridinium trifluoroacetate,[38] and 74 mg of dicyclohexylcarbodiimide in 1 ml of dimethyl sulfoxide and 1 ml of ether was stirred at room temperature overnight.[9] The reaction mixture was poured into water, extracted with ether, and the ethereal extract was washed with dilute hydrochloric acid, water, 5% sodium bicarbonate, and water. Drying over anhydrous magnesium sulfate, followed by removal of solvent under reduced pressure, provided a froth, which was subjected to column chromatography, using 4.5 g of netural alumina, activity II. Elution with *n*-hexane gave 22 mg of Δ^{12}-oleanen-28-al (V): mp 165–168°, after recrystallization from methanol–chloroform; $\lambda_{max}^{CHCl_3}$ 3.69 μ (w), 5.80 μ (s). Increasing the eluent polarity to 1:2 *n*-hexane–benzene afforded 19 mg of starting material IV. *Anal.* Calcd for $C_{30}H_{48}O$: mol wt, 424. Found by mass spectrometry: mol wt, 424.

Δ^{12}-Oleanen-28-al Ethane Dithioacetal (VI).—A solution of 59 mg of the aldehyde V, 0.5 ml of ethanedithiol, and 2 ml of boron trifluoride–ether complex in 8 ml of glacial acetic acid was stirred at room temperature overnight. The resulting solution was then poured into water, extracted with ether, and the ether extract was washed with dilute, aqueous sodium hydroxide (eight times) and with water. After drying over anhydrous magnesium sulfate, the organic solvent was evaporated under diminished pressure to leave an oil, which was chromatographed over 8 g of neutral alumina, activity II. The *n*-hexane eluate afforded 54 mg of Δ^{12}-oleanen-28-al ethanedithioacetal (VI), which did not exhibit carbonyl absorption in the infrared spectrum. Recrystallization from methanol–chloroform produced crystals of mp 173.5–175°. *Anal.* Calcd for $C_{32}H_{52}S_2$: C, 76.80; H, 10.40; mol wt, 500. Found: C, 76.52; H, 10.38; mol wt (mass spectrometry), 500.

Δ^{12}-Oleanene-28,28-d_2 (VII).—A mixture of 30 mg of the dithioacetal VI and *ca.* 1 g of deuterium-containing Raney nickel[10] in 10 ml of O-deuteriomethanol was maintained at reflux overnight while under nitrogen. The catalyst was filtered by passing the reaction mixture over activity III alumina and the solvent evaporated under reduced pressure to yield a colorless powder. The latter was taken up in ether and the ethereal solution was washed with water and dried over anhydrous magnesium sulfate. Removal of solvent under vacuum afforded a glass which was chromatographed over 2 g of neutral alumina, activity II, using *n*-hexane as eluent, to provide 20 mg of Δ^{12}-oleanene-28,28-d_2: mp 162–164° after recrystallization from methanol–chloroform; mass spectrum: *m/e* 410 (d_0), 7%; *m/e* 411 (d_1), 24%; *m/e* 412 (d_2), 63%; *m/e* 413 (d_3), 6%.

Δ^{12}-Oleanen-30-ol (XIII).—In practice it was found easiest not to isolate the carboxylic acid intermediates in the transformation IX → X → XI → XII → XIII.

A solution of 400 mg of methyl deoxyglycyrrhetate (IX)[12] in 70 ml of 10% potassium hydroxide in methanol was heated under reflux for 22 hr. The cooled solution was poured into water, acidified with 18 ml of concentrated hydrochloric acid, and extracted with ether. The ethereal extracts were washed thrice with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent afforded a froth (X) which was converted to the desired alcohol XIII by chromic acid oxidation[7] to the keto acid XI followed by Huang-Minlon reaction[8] to the acid XII and subsequent lithium aluminum hydride reduction as previously described. Recrystallization from methanol–chloroform afforded pure Δ^{12}-oleanen-30-ol XIII: mp 173–175° (lit.[39] mp 178°).

Δ^{12}-Oleanen-30-al (XIV).—Oxidation of 66 mg of XIII to the aldehyde XIV was performed according to the procedure of Pfitzner andMoffat[9] as described for the similar preparation of Δ^{12}-oleanen-28-al (V). This method produced 28 mg of Δ^{12}-oleanen-30-al (XIV): mp 129–133° after a single recrystallization from methanol–chloroform; $\lambda_{max}^{CHCl_3}$ 3.69 μ (w), 5.80 μ (s); $[\alpha]^{26.0}D$ +110.2° (*c* 0.49). *Anal.* Calcd for $C_{30}H_{48}O$: C, 84.90; H, 11.32; mol wt, 424. Found: C, 84.63; H, 11.38; mol wt (mass spectrometry), 424.

Δ^{12}-Oleanene-30,30-d_2 (XVI).—The aldehyde XIV (22 mg) was converted to its ethandithioacetal and the latter, without purification, was desulfurized to Δ^{12}-oleanene-30,30-d_2 (XVI) by using deuterium-containing Raney nickel[10] as previously described. Chromatography over 1 g of neutral alumina, activity I, using *n*-hexane as eluent, furnished 8 mg of pure XVI: mp 156–158°, after recrystallization from methanol–chloroform; mass spectrum: *m/e* 410 (d_0), 7%; *m/e* 411 (d_1), 19%, *m/e* 412 (d_2), 62%; *m/e* 413 (d_3), 12%.

Δ^{12}-Oleanen-3-one (XVIII).—Jones oxidation[7] of β-amyrin (XVII), mp 197–199°, as previously described afforded β-amyrone (XVIII) in quantitative yield, mp 176–178° after recrystallization from methanol–chloroform (lit.[40] mp 178–180°).

Δ^{12}-Oleanene-3,3-d_2 (XX).—A mixture of 100 mg of β-amyrone (XVIII), 100 mg of *p*-toluenesulfonylhydrazide, and 4 drops of acetyl chloride in 6 ml of methanol was heated to reflux temperature and dioxane was added dropwise until a homogeneous solution resulted. The latter was maintained at reflux overnight, cooled, poured into water, and the organic material was extracted with ether. The ethereal extract was washed with water, dilute hydrochloric acid, water, 5% sodium bicarbonate, and water, and dried over anhydrous magnesium sulfate. Removal of solvent under diminished pressure afforded an oil which by thin layer chromatographic analysis was shown to consist of the expected tosylhydrazone (XIX) and the starting ketone. This mixture was not separated but reduced directly with lithium aluminum deuteride.[13]

To a stirred suspension of 150 mg of lithium aluminum deuteride in dry dioxane (freshly distilled from sodium) was added dropwise a solution of the crude tosylhydrazone XIX in the same solvent. The resulting suspension was heated under reflux for 2 hr, while being kept under an atmosphere of nitrogen. The reaction mixture was cooled in an ice–water bath and deuterium oxide was added cautiously dropwise. Excess anhydrous magnesium sulfate was added and the suspension was filtered. The filter cake was washed well with ether. The filtrate was washed with water, 5% sodium bicarbonate (twice), and water, dried over anhydrous magnesium sulfate, and evaporated to dryness. A thin layer chromatogram indicated that the residue was a mixture of alcohol XVII (containing a 3α deuterium atom) and hydrocarbon compounds, by comparison with thin layer chromatographic mobilities of authentic material. Chromatography over 1 g of neutral alumina, activity II, using *n*-hexane as eluent afforded the nonpolar product, which by thin layer chromatographic analysis, using a 5% silver nitrate impregnated silica gel G plate,[41] was shown to consist of XX (by direct comparison with authentic nondeuterated material) and a less polar compound (probably $\Delta^{2,12}$-oleadiene and ring-contracted material[42]). The desired Δ^{12}-oleanene-3,3-d_2 (12 mg) was isolated by preparative thin layer chromatography using a 5% silver nitrate impregnated silica gel H chromatoplate:[41] mp 156–158° after recrystallization from methanol–chloroform; mass spectrum: *m/e* 410 (d_0), 9%; *m/e* 411 (d_1), 42%; *m/e* 412 (d_2), 49%.

Δ^{12}-Oleanen-11-one (XXII).—To a boiling solution of 300 mg of Δ^{12}-oleanene (XXI)[43] in 18 ml of glacial acetic acid was added dropwise a solution of 425 mg of chromium trioxide in 15 ml of 85% acetic acid[14] and the resulting solution stirred vigorously and heated under reflux for 2 hr. The cooled solution was poured into water, extracted with ether, and the ethereal extract was washed with water, dilute sodium hydroxide solution, and water. After drying over anhydrous magnesium sulfate, the solvent was evaporated under diminished pressure to provide a colorless solid. The crude product was chromatographed over 30 g of silica gel (Grace-Davison Chemical Co., Baltimore, Md). *n*-Hexane elution furnished 30 mg of starting material (XXI), while increasing the eluent polarity to 4:1 *n*-hexane–benzene gave a small amount of by-product (not characterized). Further elution

(38) Prepared by adding 2 ml of trifluoroacetic acid dropwise to a stirred solution of 4.5 ml of pyridine in 15 ml of ether. The white solid which results is collected by filtration, washed with ether and used immediately.

(39) O. Jeger and W. Hofer, *Helv. Chim. Acta*, **31**, 157 (1948).

(40) G. Brownlie, M. B. E. Fayez, F. S. Spring, R. Stevenson, and W. S. Strachan, *J. Chem. Soc.*, 1377 (1956).

(41) A. S. Gupta and S. Dev, *J. Chromatog.*, **12**, 189 (1963).

(42) R. H. Shapiro, *Tetrahedron Letters*, in press.

(43) E. J. Corey and J. J. Ursprung, *J. Am. Chem. Soc.*, **78**, 5041 (1956).

with 2:1 *n*-hexane–benzene afforded 210 mg of XXII. Two recrystallizations from methanol–chloroform gave pure Δ^{12}-oleanen-11-one: mp 214–216°; λ_{max}^{EtOH} 250 mμ (ε 12,600); $\lambda_{max}^{CHCl_3}$ 6.08 μ (s), 6.17 μ (m); ORD (*c* 0.10, in dioxane): $[\phi]_{589}$ +957°, $[\phi]_{390}$ +3830°, $[\phi]_{380}$ +3447°, $[\phi]_{369}$ +4404°, $[\phi]_{362}$ +4021° $[\phi]_{352}$ +5936°, $[\phi]_{332}$ +7758°, $[\phi]_{254}$ +42,130°. *Anal.* Calcd for $C_{30}H_{48}O$: mol wt, 424. Found by mass spectrometry: mol wt, 424.

Δ^{12}-Oleanene-11,11-d_2 (XXIII).—A suspension of 30 mg of platinum oxide in O-deuterioacetic acid[15] was prereduced with deuterium gas and a solution of 18 mg of the α,β-unsaturated ketone XXII in O-deuterioacetic acid was added. After stirring the suspension in the presence of deuterium gas for 5 days, the catalyst was filtered and the filtrate was poured into water, extracted with ether, and the ethereal solution was washed with 5% $NaHCO_3$ (twice) and water (three times). The dried solution was evaporated to dryness, leaving 15 mg of a colorless solid shown to consist of a mixture of starting material and desired product by thin layer chromatographic analysis. Chromatography of the crude reaction product over 1 g of neutral alumina, activity I, and methanol–ether recrystallization of the material obtained from the first two *n*-hexane fractions afforded Δ^{12}-oleanene-11,11-d_2: mp 158–160°. The mass spectrum of this substance exhibited a weak molecular ion region, precluding accurate purity determination of isotopic purity from the M^+ region. The isotopic distribution was calculated from the *m/e* 220 area (retro-Diels–Alder fragment) and was found to be 3% d_0, 17% d_1, and 80% d_2.

18α-Δ^{12}-Oleanen-11-one (XXIV).—A solution of 172 mg of Δ^{12}-oleanen-11-one (XXII) in 25 ml of 15% potassium hydroxide in absolute ethanol was heated under reflux under nitrogen for 52 hr.[14] The cooled solution was poured into water, extracted with ether, and the ethereal extract was washed with water (twice), dilute hydrochloric acid, and water. The dried solution was evaporated to dryness under vacuum, leaving a yellow amorphous material. The crude product was subjected to a careful gradient elution chromatography over 23 g of silica gel (Grace-Davison Chemical Co., Baltimore, Md.). Elution with 10% *n*-hexane–benzene gave 27 mg of the desired product XXIV (fractions 38–55) and 54 mg of a mixture of 18α and 18β isomers (fractions 56–88). Increasing the eluent polarity to 20% hexane–benzene provided an additional 51 mg of a mixture of the isomeric α,β-unsaturated ketones. The impure fractions were combined and the chromatographic procedure repeated to afford an additional 20 mg of desired product XXIV. Pure 18α-Δ^{12}-oleanen-11-one was recrystallized from methanol–chloroform: mp 181.5–182°; λ_{max}^{EtOH} 245 mμ (ε 10,920); $\lambda_{max}^{CHCl_3}$ 6.02 (s), 6.12 μ (m); ORD (*c* 0.11, in dioxane), $[\phi]_{589}$ +391°, $[\phi]_{375}$ −2266°, $[\phi]_{365}$ −1016°, $[\phi]_{360}$ −1407°, $[\phi]_{255}$ +22,158°, $[\phi]_{240}$ +8441°.

18α-Δ^{12}-Oleanen-11-one-28,28-d_2 (XXVI).—Allylic oxidation of VII (isotopic purity: 14% d_0, 27% d_1, 51% d_2, and 8% d_3) to the enone XXV and subsequent base equilibration to form XXVI was performed as previously described for the analogous sequence with nondeuterated compounds. The d_2 analog XXVI upon recrystallization from ethanol–chloroform exhibited mp 281.5–283°; $\lambda_{max}^{CHCl_3}$ 6.02 (s), 6.12 μ (m). *Anal.* Calcd for $C_{30}H_{46}D_2O$: C, 84.51; H + D, 11.74. Found: C, 84.33; H + D, 11.59.

$\Delta^{11,13(18)}$-Oleadiene-28,28-d_2 (XXVIII).—Lithium aluminum hydride reduction, as previously described, of XXVI afforded 18α-Δ^{12}-oleanen-11β-ol-28,28-d_2 (XXVII),[18] which was not isolated but directly subjected to the following reaction conditions.

A suspension of 28 mg of the crude allylic alcohol XXVII and 20 mg of platinum oxide in glacial acetic acid was stirred in the presence of hydrogen gas for 24 hr. The catalyst was filtered and the solution was evaporated to dryness. The residue was chromatographed over 1 g of neutral alumina, activity II, using *n*-hexane as eluent to provide 25 mg of colorless material, homogenous on a silver nitrate impregnated[41] thin layer chromatogram. Recrystallization from methanol–chloroform afforded crystals melting at 203–206°; λ_{max}^{EtOH} 242 mμ (ε 23,000), 250 mμ (ε 25,800), 259 mμ (ε 17,500). *Anal.* Calcd for $C_{30}H_{46}D_2$: mol wt, 410. Found by mass spectrometry: mol wt, 410.

18α-Δ^{12}-Oleanene-28,28-d_2 (XXIX).—To a blue solution of *ca.* 20 mg of lithium metal in 5 ml of freshly distilled ethylamine was added a solution of 55 mg of the crude allylic alcohol XXVII in tetrahydrofuran[20] (freshly distilled from lithium aluminum hydride). The reaction was continued for 5 min while using a Dry Ice–isopropyl alcohol condenser to contain the ethylamine. Excess lithium was decomposed by dropwise addition of methanol at 0° and the resulting colorless mixture was warmed gently to remove the organic solvent. The residue was taken up in ether–water and the ethereal solution was washed with water, dilute hydrochloric acid, water, 5% sodium bicarbonate, and water. After drying over anhydrous magnesium sulfate, the solvent was evaporated under diminished pressure, leaving an oil which was chromatographed over 3 g of neutral alumina, activity I. Elution with *n*-hexane afforded 40 mg of 18α-Δ^{12}-oleanene-28,28-d_2: mp 187.5–189.5° after recrystallization from methanol–chloroform; mass spectrum: *m/e* 410 (d_0), 17%; *m/e* 411 (d_1), 28%; *m/e* 412 (d_2), 50%; *m/e* 413 (d_3), 5%.

Δ^{12}-Ursen-28-ol (XXXIII).—Ursolic acid was converted to XXXIII in three steps as described above for the similar transformation in the Δ^{12}-oleanene series. However, in the present case the primary alcohol XXXIII could not be induced to crystallize. The amorphous material which was obtained exhibited a melting point range of 77–89°, $[\alpha]^{27}D$ +72.64° (*c* 1.0) *Anal.* Calcd for $C_{30}H_{50}O$: mol wt, 426. Found by mass spectrometry: mol wt, 426.

Δ^{12}-Ursen-28-al (XXXIV).—Moffat oxidation,[9] as previously described, of 274 mg of Δ^{12}-ursen-28-ol (XXXIII) resulted in the isolation of 120 mg of XXXIV: mp 96–102° after recrystallization from aqueous methanol (lit.[44] mp 172–174°); $\lambda_{max}^{CHCl_3}$ 5.80 (s), 3.69 μ (w). *Anal.* Calcd for $C_{30}H_{48}O$: mol wt, 424. Found by mass spectrometry: mol wt, 424.

Δ^{12}-Ursen-28-al Ethane Dithioacetal (XXXV).—Prepared as described in the preparation of Δ^{12}-oleanen-28-al ethane dithioacetal (VI). The crude product obtained from reaction of 105 mg of aldehyde XXXIV, when subjected to chromatography over 20 g of neutral alumina, activity II, using *n*-hexane as eluent afforded 90 mg of XXXV, mp 95–98° after recrystallization from methanol–chloroform. *Anal.* Calcd for $C_{32}H_{52}S_2$: mol wt, 500. Found by mass spectrometry: mol wt, 500.

Δ^{12}-Ursene-28,28-d_2 (XXXVI).—Desulfurization of 57 mg of XXXV with deuterium-containing Raney nickel in O-deuteriomethanol as previously described provided 23 mg of Δ^{12}-ursene-28,28-d_2 (XXXVI). Two recrystallizations from methanol–ether gave crystals melting at 105–109°; mass spectrum: *m/e* 410 (d_0), 7%; *m/e* 411 (d_1), 23%; *m/e* 412 (d_2), 62%; *m/e* (d_3), 8%.

Δ^{12}-Ursen-3β-ol Benzoate-12,27-d_2 (XLII).—A solution of 195 mg of phyllanthyl benzoate[22] in 7 ml of alcohol-free chloroform was placed in a 25-ml three-necked flask equipped with a gas inlet tube and connected to a water aspirator.[21] Deuterium chloride gas was generated by the dropwise addition of distilled phosphorus trichloride to stirred deuterium oxide, maintained at room temperature in a 100-ml three-necked flask which was connected by tygon tubing to a calcium chloride tube and the latter joined to the smaller three-necked flask by tygon tubing. The reaction vessel was partially evacuated and gaseous deuterium chloride admitted. This evacuation and deuterium chloride addition was repeated two additional times, the reaction vessel was closed, and the solution was stirred at room temperature overnight. Evaporation of solvent afforded a quantitative yield of deuterated α-amyrin benzoate (XLII), mp 196.5–199° after recrystallization from methanol–chloroform. For isotopic purity, see compound XLV.

Δ^{12}-Ursen-3β-ol-12,27-d_2 (XLIII).—Lithium aluminum hydride reduction, as mentioned earlier, converted 180 mg of the benzoate (XLII) to 140 mg of the free 3β alcohol (XLIII). This product, upon recrystallization from methanol, exhibited a melting point of 186–188°.

Δ^{12}-Ursen-3-one-12,27-d_2 (XLIV).—The usual Jones oxidation[7] of 110 mg of XLIII gave 100 mg of the deuterated α-amyrone (XLIV), mp 124–125° after a single recrystallization from methanol.

Δ^{12}-Ursene-12,27-d_2 (XLV).—The previously described Huang-Minlon reduction of 70 mg of XLIV afforded 61 mg of the isotopically labeled hydrocarbon XLV: mp 111–117° after recrystallization from methanol–chloroform; mass spectrum: *m/e* 410 (d_0), 20%; *m/e* 411 (d_1), 35%; *m/e* 412 (d_2), 42%; *m/e* 413 (d_3), 3%.

Δ^{12}-Ursene (XLI).—Conversion of phyllanthyl benzoate (XXXVII)[21,23] to phyllanthane (XL) was accomplished by the lithium aluminum hydride reduction–Jones oxidation[7]–Huang-Minlon reduction[8] route discussed above. Opening of the cyclopropane ring was affected according to literature directions[21] by

(44) J. Polonsky, *Compt. Rend.*, **233**, 671 (1951).

heating a solution of 40 mg of phyllanthane in 10 ml of acetic acid and 1 ml of concentrated hydrochloric acid at reflux temperature. The cooled reaction was poured into water, extracted with ether, and the ethereal extract was washed with water, 5% sodium bicarbonate, and water. After drying over anhydrous magnesium sulfate, the solvent was evaporated under reduced pressure to afford an oil which was chromatographed over 1 g of neutral alumina, activity II. Elution with *n*-hexane gave 15 mg of Δ^{12}-ursene (XLI), mp 100–105° after recrystallization from methanol–chloroform.

Stereoselective Syntheses of Optically Active Amino Acids from Menthyl Esters of α-Keto Acids[1]

KAZUO MATSUMOTO AND KAORU HARADA

Institute of Molecular Evolution and Department of Chemistry, University of Miami, Coral Gables, Florida

Received January 6, 1966

Menthyl esters of pyruvic acid, α-ketobutyric acid, and phenylglyoxylic acid were converted to their oximes and Schiff bases of benzylamine. These were hydrogenated catalytically by the use of palladium on charcoal and palladium hydroxide on charcoal. Optically active D-alanine (optical yield 16–25%), D-α-aminobutyric acid (8–21%), and D-phenylglycine (44–49%) were obtained. Possible steric courses of the reactions are discussed.

l-Menthol has been used as an optically active moiety in many types of stereochemical studies.[2] In previous studies the menthyl esters of various α-amino acids have been synthesized by the azeotropic method[3] and from α-amino acid N-carboxyanhydrides.[4]

In this study, the N-hydroxyimino (oxime) and N-benzylimino (benzylamine Schiff base) derivatives of menthyl pyruvate, menthyl α-ketobutyrate, and menthyl phenylglyoxylate were synthesized. These were hydrogenated and hydrogenolyzed by the use of palladium on charcoal (catalyst A) or palladium hydroxide on charcoal[5] (catalyst B) to yield the menthyl esters of alanine, α-amino-*n*-butyric acid, and of phenylglycine. These asymmetrically synthesized menthyl esters of amino acids were saponified by alkali in aqueous alcohol.[6] The liberated, free amino acids were isolated and their optical activities measured to determine the optical yield. However, the isolation and recrystallization procedure resulted in fractionation of the optically active amino acids. The specific rotation of amino acids decreased upon recrystallization and finally the value reached zero after several purification procedures, To avoid the fractionation and to determine the accurate optical purity of the synthesized amino acids, a part of the hydrolyzed reaction mixture was directly treated with 1-fluoro-2,4-dinitrobenzene to yield dinitrophenylamino acids.[7] The resulting DNP-amino acids were isolated chromatographically by the use of a Celite column treated with pH 7 phosphate–citrate buffer.[8] The DNP-amino acids thus obtained were analytically pure without further purification. An advantage of the DNP method was to avoid fractionation completely during the isolation and purification procedures.

Table I shows the summarized results which were obtained by the catalytic hydrogenation procedure. Partially optically active (8–49%) D-amino acids were obtained. However, the optical activities of the amino acids prepared by the use of palladium on charcoal (catalyst A) from the Schiff base of pyruvate and of α-ketobutyrate were found to be zero. The optical activity of α-aminobutyric acid prepared by the use of palladium hydroxide on charcoal (catalyst B) was also zero; however, the DNP-α-aminobutyric acid showed optical activity, $[\alpha]^{25}D$ −7.3°. The latter case can be explained by the fractionation of the product during the isolation procedure. The results show that the hydrogenation reaction of hydroxyimino derivatives (oxime) gave higher optical activity than that of benzylimino derivatives (Schiff base). Menthyl phenylglyoxylate did not form the Schiff base with benzylamine under the azeotropic distillation method which was employed for the other keto esters.

To check the racemization of amino acids during the saponification by alkali, the authentic menthyl esters of D-alanine, D-α-aminobutyric acid, and D-phenylglycine[3,4] were hydrolyzed by the same procedure employed in the hydrolysis of the menthyl esters synthesized in this study. Optical rotations were measured as DNP-amino acid which was separated by column chromatography. Racemization of D-alanine and D-α-aminobutyric acid was slight (4 and 3%, respectively); however, D-phenylglycine lost 77% of its activity during the saponification procedure. The optical purities listed in Table I are corrected by use of the values of standard racemization.

The steric course of the synthesis could be explained in a way similar to the rules proposed by Cram[9] and Prelog[10] as is shown in Scheme I. The most stable conformation might be structure I since C=O and C=N groups repel each other because of their electric dipoles. The menthyl residue is considered to take a conformation as was proposed by Prelog[10] (Scheme I). The molecules would be absorbed with the less bulky side on a catalyst, and the hydrogen atoms would attack

(1) Sterically Controlled Syntheses of Optically Active Organic Compound. I. Contribution No. 059 of the Institute of Molecular Evolution, University of Miami.

(2) A. McKenzie, *J. Chem. Soc.*, **85**, 1249 (1904); A. McKenzie and H. B. P. Humphries, *ibid.*, **95**, 1105 (1909); A. McKenzie and I. A. Smith, *Ber.*, **58**, 899 (1925); V. Prelog, *Helv. Chim. Acta*, **36**, 308 (1953); H. M. Walborskey, *et al.*, *J. Am. Chem. Soc.*, **81**, 1514 (1959); **83**, 2517 (1961); Y. Inoue, *et al.*, *ibid.*, **82**, 5255 (1960).

(3) K. Harada and T. Hayakawa, *Bull. Chem. Soc. Japan*, **37**, 191 (1964).

(4) T. Hayakawa and K. Harada, *ibid.*, **38**, 1354 (1965).

(5) R. G. Hiskey and R. C. Northrop, *J. Am. Chem. Soc.*, **83**, 4798 (1961).

(6) DL-Amino acid menthyl esters were fractionated easily during the isolation and recrystallization procedure;[3,4] it was found difficult to estimate the optical yield by isolation of the menthyl esters without fractionation.

(7) F. Sanger, *Biochem. J.*, **39**, 507 (1945); F. C. Green and C. M. Kay, *Anal. Chem.*, **24**, 726 (1952); K. R. Rao aud H. A. Sober, *J. Am. Chem. Soc.*, **76**, 1328 (1954).

(8) J C. Perrone, *Nature*, **167**, 513 (1951); A. Courts, *Biochem. J.*, **58**, 70 (1954).

(9) D. J. Cram and F. A. Abd Elhafez, *J. Am. Chem. Soc.*, **74**, 5828 (1952).

(10) V. Prelog, *Helv. Chim. Acta*, **36**, 308 (1953).