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STEROIDS AND RELATED NATURAL PRODUCTS—XXVII.

*SALVIA APIANA**

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Abstract—The pentacyclic triterpene composition of a southern California variety of *Salvia apiana* (fam. Labiatae) has been assessed. The presence of α-amyrin, oleanolic acid and ursolic acid has been established. Separation of α-amyrin from a closely related impurity believed to be β-amyrin was accomplished by subjecting the mixture to selenium dioxide oxidation and isolating α-amyrin by preparative thin-layer chromatography on silver nitrate-silica gel. Evidence for the presence of isomeric mixtures of two dihydroxy triterpenes and two triterpene aldehydes representing intermediate stages of oxidation from α- and β-amyrin to ursolic and oleanolic acids was presented. Isolation of ursolic acid from a Mexican variety of *Salvia karwinskii* was also reported.

MANY members of the Labiatae† family have been employed as flavoring agents, spices, in the manufacture of perfumes and in primitive medical practice.[1] Phytochemical studies of this family have dealt primarily with economically important members of the lavender (*Lavandula*), mint (*Mentha*), sage (*Salvia*) and rosemary (*Rosmarinus*) genera.[2] The family is rich in essential oil-bearers, which contain a variety of mono-, sesqui- and diterpenes.[3] Also,

* Communications regarding this manuscript should be sent to G.R.P., Dept. of Chemistry, Arizona State University, Tempe, Arizona. Part XXVI, G. R. PETTIT and D. M. PIATAK, *Canad. J. Chem.* In press.

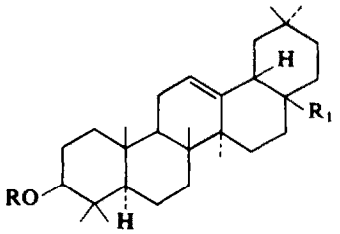
† Variously known as Lamiaceae, Menthaceae or mints, the Labiatae family is composed of 3200 to 3300 species (grouped in 160–200 genera) of wide geographical distribution in temperate and tropical regions. Most of these species are aromatic, square-stemmed herbaceous annuals or perennials, while some tropical species are trees.

[1] A comprehensive survey of Labiatae used in folk medicine has been prepared by G. DRAGENDORFF, *Die Heilpflanzen*, p. 568. Verlag von Ferdinand Enke, Stuttgart (1898). An interesting collection of medicinal and poisonous Labiatae of India has been summarized by J. F. CAIUS, *J. Bombay Nat. Hist. Soc.* **42**, 380 (1941). Another review pertinent to *Salvia* species appears in the M.S. thesis submitted by N. N. Jorgensen to the Graduate School, University of Maine, August, 1960. The toxic properties of certain Labiatae members have also been described by: C. A. GARDNER and H. W. BENNETTS, *The Toxic Plants of Western Australia*, p. 161. West Australia Newspapers (1956); and L. J. WEBB, *Guide to the Medicinal and Poisonous Plants of Queensland*, Council for Scientific and Industrial Research, Bull. No. 232, p. 67 (1948).

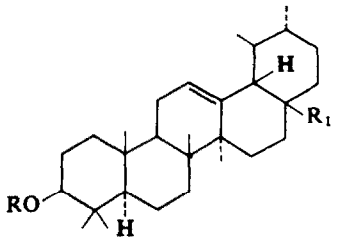
[2] (*a*) See, for example: C. H. BRIESKORN and E. WENGER, *Arch. Pharm.* **293**, 21 (1960); (*b*) R. H. REITSEMA, *J. Am. Pharm. Assoc.* **47**, 265 (1958); and (*c*) M. CALVARANO, *Essenze Deriv. Agrumari* **27**, 208 (1957) [*Chem. Abstr.* **52**, 10,507 (1958)].

[3] The valuable and extensive investigations of *Salvia officinalis* and *carnosa* by C. H. BRIESKORN and colleagues provide examples in the *Salvia* genus: (*a*) C. H. BRIESKORN and W. POLONIUS, *Die Pharmazie* **11**, 705 (1962); (*b*) C. H. BRIESKORN, A. FUCHS, J. B. BREDENBERG, J. D. MCCHESNEY and E. WENKERT, *J. Am. Chem. Soc.* **29**, 2293 (1964); and (*c*) C. H. BRIESKORN and S. DALFERTH, *Ann. Chem.* **676**, 171 (1964). Two new diterpenes have recently been isolated from *Salvia officinalis* by H. LINDE, *Helv. Chim. Acta* **47**, 1234 (1964).

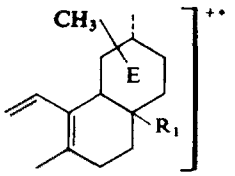
the pentacyclic triterpenes oleanolic acid (Ia) and/or ursolic acid (IIa) have been identified in species representing approximately 20 genera.[4] Widespread distribution of the α- and β-amyrin acids combined with an abundant supply of possible mono- and diterpene biogenetic precursors suggested that the Labiatae family might contain a variety of new and perhaps novel pentacyclic triterpenes.* With a long-term view toward providing information of potential use in solution of biogenetic and taxonomic problems[5] involving the Labiatae family, we began in 1958 to evaluate the triterpene composition of various Labiatae species.†



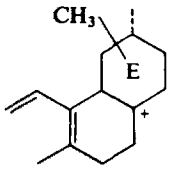
Ia, R = H, R_1 = CO_2H
b, R = H, R_1 = CH_3
c, R = H, R_1 = CH_2OH
d, R = H, R_1 = CHO
e, R = $OCCH_3$, R_1 = CHO



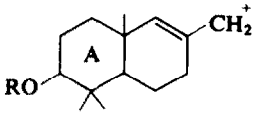
IIa, R = H, R_1 = CO_2H
b, R = H, R_1 = CH_3
c, R = H, R_1 = CH_2OH
d, R = H, R_1 = CHO
e, R = $OCCH_3$, R_1 = CHO



IIIa, R_1 = CH_3, *m/e* 218
b, R_1 = CH_2OH, *m/e* 234
c, R_1 = CHO, *m/e* 232



IV *m/e* 203



Va, R = H, *m/e* 207
b, R = $OCCH_3$, *m/e* 249

Of several Labiatae species initially examined for triterpene components, a southern California variety of *Salvia apiana* appeared most promising and was first selected for detailed study.‡ The dry foliage of *Salvia apiana*, a spicy-odored, gray perennial, was

* Recently, isolation of germanicol from *Salvia officinalis* has been reported [refer to Ref. 3 (*a*)].

† Selection of the Labiatae family was also based on the observation that it represented one of several plant families whose members have been most frequently employed in primitive treatment of neoplastic disease. We are indebted to Dr. Ronald B. Ross, National Institutes of Health, Public Health Service, for bringing this information to our attention and for his generous assistance in obtaining a number of rare Labiatae species.

‡ The *Salvia* genus generally resides in tropical and temperate regions and encompasses approximately 550 species. One of these, *Salvia officinalis* (Ref. 3(*a*)), has been examined for pentacyclic triterpene constituents, while approximately 20 other *Salvia* species have been studied in respect to other types of natural products. No prior phytochemical study of *Salvia apiana* has been reported.

[4] H. POURRAT and J. LEMEN, *Ann. Pharm. Franc.* **11**, 190 (1953); C. H. BRIESKORN, K. H. EBERHARDT and M. BRINER, *Arch. Pharm.* **286**, 501 (1953); C. H. BRIESKORN, M. BRINER, L. SCHLUMPRECHT and K. H. EBERHARDT, *Arch. Pharm.* **285**, 291 (1952); and E. J. ROWE, J. E. ORR, A. H. UHL and L. M. PARKS, *J. Am. Pharm. Assoc.* **38**, 122 (1949).

[5] A biosynthetic scheme to account for relationships between several monoterpene components of the mint genus has been proposed by R. H. REITSEMA, *J. Am. Pharm. Assoc.* **47**, 267 (1958). The general area of taxonomy based on chemically recognized constituents has been summarized recently in a text edited by T. SWAIN, *Chemical Plant Taxonomy*. Academic Press, New York (1963).

successively extracted with hexane, diethyl ether and 95% ethanol. A variety of procedures were evaluated for isolating triterpene components. The following method, starting with 2·8 kg of plant material and using the Liebermann–Burchard reaction[6] to monitor separation, proved most convenient.

A methanol-soluble portion of the hexane extract was saponified and neutral components were partially separated by column chromatography on silica gel. Each fraction containing triterpenes or sterols (as indicated by the Liebermann–Burchard test) was further purified by rechromatography on neutral alumina and/or by fractional recrystallization. By this means, a substance (0·18 g) with properties similar to β-sitosterol[7] was isolated in addition to monohydroxy (0·7 g) and dihydroxy triterpenes (0·6 g), and a triterpene aldehyde (0·5 g). On the basis of elemental composition, mass and i.r. spectral data, and chemical behavior, the triterpenes appeared to be mixtures of oleanene (I) and ursene (II) derivatives. From the monohydroxy triterpene mixture was isolated α-amyrin (IIb) and the acetyl derivative was found to be identical with an authentic sample.[8] Furthermore, the mass spectral fragmentation pattern of α-amyrin displayed peaks at *m/e* 218 (IIIa), 203 (IV) and 207 (Va) as predicted by Djerassi and colleagues.[9] Selenium dioxide[10] oxidation of an impure sample of the α-amyrin acetate provided both a pure specimen and evidence that the closely related impurity was very likely β-amyrin (Ib). The oxidation product exhibited one spot on a silica gel thin-layer chromatogram but was readily resolved into a pure sample of α-amyrin acetate and a diene impurity by a preparative thin-layer technique[11a] employing silica gel treated with silver nitrate. The small quantity (12 mg) of impure diene gave the expected[11b] u.v. absorption curve with extinction coefficients about 10 per cent less than those recorded for the diene derived from β-amyrin acetate.

Physical constants for the dihydroxy triterpene did not agree with either of those reported for erythrodiol (Ic) or uvaol (IIc) and it was found to be a mixture of at least two substances by the selenium dioxide technique outlined for α-amyrin acetate. However, the oxidation products resisted final purification; characterization of a constant melting (213–215°) sample of the dihydroxy triterpenes as 3,28-dihydroxy-Δ^{12}-oleanene and -ursenes was readily achieved by mass spectral analysis. Predictable[9] fragments were found at *m/e* 234 (IIIb), 203 (IV) and 207 (Va).

The oily triterpene aldehyde was converted to a crystalline acetate derivative and the latter was reduced by lithium aluminum hydride to a mixture corresponding to the triterpene diols noted above. Oxidizing the acetoxy-triterpene aldehyde, employing an 8N chromium trioxide reagent[12] in acetone solution, gave a mixture of products one of which showed the same mobility on a thin-layer chromatogram as 3β-acetoxy oleanolic and/or ursolic acids.

[6] A useful procedure for quantitatively evaluating composition of oleanolic-ursolic acid mixtures based on the Liebermann–Burchard reaction has been developed by C. H. BRIESKORN, *Arch. Pharm.* **295**, 505 (1962).

[7] Difficulties inherent in adequately characterizing β-sitosterol have been summarized by G. I. FUJIMOTO and A. E. JACOBSON, *J. Org. Chem.* **29**, 3377 (1964). A biosynthetic study of β-sitosterol in *Salvia officinalis* has been reported by H. J. NICHOLAS and S. MORIARTY, *Federation Proc.* **22**, Part I, 529 (1963): See also, M. CASTLE, G. BLONDIN and W. R. NES, *J. Am. Chem. Soc.* **85**, 3306 (1963).

[8] E. J. COREY and E. W. CANTRALL, *J. Am. Chem. Soc.* **81**, 1745 (1959).

[9] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963); H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. 2, p. 121 Holden-Day, San Francisco, (1964).

[10] G. H. STOUT and K. L. STEVENS, *J. Org. Chem.* **28**, 1259 (1963).

[11] (*a*) For example see, J. W. COPIUS-PEEREBOOM and H. W. BEEKES, *J. Chromatog.* **17**, 99 (1965); and C. B. BARRETT, M. S. J. DALLAS and F. B. PADLEY, *Chem. & Ind. (London)* 1050 (1962); (*b*) R. BUDZIAREK, W. MANSON and F. S. SPRING, *J. Chem. Soc.* 3336 (1951).

[12] K. BOWDEN, I. HEILBRON, E. R. H. JONES and B. C. L. WEEDON, *J. Chem. Soc.* 39 (1946).

Again, mass spectrometric methods proved most useful for characterizing the acetoxy-triterpene aldehyde mixture. A specimen of the acetoxy-triterpene aldehyde, displaying one spot on a thin-layer chromatogram, showed ion peaks (IIIc, IV and Vb) expected for a 3-acetoxy-28-oxo-Δ^{12}-oleanene or -ursene (e.g. Ie or IIe). As with α-amyrin and the diol mixture, another peak at *m/e* 189 corresponding to, e.g. further fragmentation by loss of acetic acid (or water) from V was also observed.

Turning now to the diethyl ether extract of *Salvia apiana*, partial removal of solvent allowed separation of a pale green solid (53 g) which consisted primarily of triterpene carboxylic acids. An attempt at crystallizing the mixture from ethanol gave a gelatinous phase reminiscent[13] of oleanolic–ursolic acid mixtures.[6,*] Extensive fractional recrystallization of the acid mixture led to a pure specimen of ursolic acid. Similar treatment of the mother liquors afforded a much smaller but pure sample of oleanolic acid. Both acids Ia and IIa were identical with authentic samples. In addition both were further characterized by conversion to acetate derivatives. Cursory examination of a similar fraction from a Mexican variety of *Salvia karwinskii*[14] again yielded ursolic acid as a major component.

Results of the present study of *Salvia apiana* triterpene components indicates that all of the major intermediates in biosynthetic oxidation sequences leading from α-amyrin to ursolic acid and from β-amyrin to oleanolic acid are represented in this species. The possibility still exists, however, that the β-amyrin accompanying α-amyrin, the triterpene diols or the triterpene aldehydes correspond with stereochemical modifications of the oleanene or ursene skeleton.

EXPERIMENTAL

General

All solvents were redistilled, and when used to extract an aqueous phase each such solution was redried over sodium sulfate. Column chromatograms were prepared employing silica gel (0·2–0·5 mm, E. Merck, A.G., Darmstadt, Germany) or activated neutral alumina (E. Merck, A.G.).

Analytical TLC was performed on 20-cm-long glass plates covered with a 0·25-mm layer of silica gel HF_{254} (E. Merck, A.G.) activated at 110°.[15] The plate was developed with antimony trichloride–chloroform spray (followed by heating at ~105° for 5 min) or by spraying with conc. H_2SO_4 and heating at ~130° for 30 min. The $SbCl_3$ method was used primarily for gross structural information and the latter spray for purity determination. The $AgNO_3$ TLC were performed using layers prepared by adding $AgNO_3$ (corresponding to 20 per cent by weight of the silica gel) to silica gel HF_{254}. Detection was accomplished with a conc. H_2SO_4. 1 mm plates were employed for preparative thin-layer chromatography, and zones were located using a water spray.

Melting points were determined with a Kofler apparatus and are uncorrected. Ultraviolet (EtOH solution) and i.r. (KBr) spectra were recorded by Dr. R. A. Hill of these laboratories. Optical rotation measurements were provided by the microanalytical laboratory of

* The recent and careful characterization of micromerol as mainly ursolic acid existing with small amounts of oleanolic acid, provides a nice illustration of such mixtures. The three-dimensional X-ray analysis of an ursolic acid derivative also described by Stout and Stevens unequivocally establishes the ring system and stereochemistry now accepted for α-amyrin (cf. Ref. 10).

[13] C. H. BRIESKORN and H. KLINGER, *Z. Lebensm. Untersuch. Forsch.* **120**, 269 (1963).

[14] While no previous phytochemical investigation of *S. karwinskii* has been described, the plant is mentioned in a botanical synopsis of Mexican and Central American species of *Salvia* prepared by M. L. FERNALD, *Contrib. Gray Herb.* **19**, 489 (1900).

[15] E. STAHL, *Chemiker Ztg.* **82**, 323 (1958).

Dr. C. Janssen, Beerse, Belgium, and elemental analyses were performed in the laboratories of Dr. A. Bernhardt, Mülheim (Ruhr), Germany. The mass spectral data was determined by J. Occolowitz and samples were volatilized close to the mass spectrometer ion source without an intervening leak.

Extraction Procedure

Preliminary phytochemical study of *Salvia apiana* was performed using a specimen of foliage collected in southern California in September, 1959, by Dr. H. S. Gentry, San Felipe Ranch, California. A variety of separation methods were explored using this material, and during this phase of the study ursolic acid was first isolated and characterized (by Dr. U. R. Ghatak). The investigation described below, however, was executed with the aerial portion of *Salvia apiana* collected in July, 1960, in southern California by Professor Orville H. Miller, University of California at Los Angeles. The plant material was dried prior to shipment at 110° using a stream of warm air. In a typical experiment, 2·8 kg of the ground stems and leaves were exhaustively extracted with hexane. Removal of solvent gave a dark green, waxy residue (165 g). After drying, the extraction procedure was repeated with diethyl ether and a greenish yellow powder (53 g) precipitated during concentration of the solution. Complete removal of solvent yielded a dark green viscous oil. This portion of diethyl ether extract and the total ethyl alcohol extract fail to provide encouraging amounts of triterpenes and were not further examined.

The Hexane Extract

The hexane extract showed a slightly positive red-violet Liebermann–Burchard (LB) test[16] indicating the presence of higher terpenes. The total extract was refluxed with methanol (3 × 1 l.) and, after cooling, the solution was filtered to remove wax-like substances. Removal of solvent from the combined methanol extract afforded 44 g of dark green residue, which was dissolved in 250 ml of methanol-KOH (10%) and refluxed for 2 hr. After removing approximately 100 ml of solvent *in vacuo*, the solution was diluted with 4% aqueous KOH (700 ml). The mixture was extracted with diethyl ether and concentrating the combined extract to dryness gave 21 g of a brown semi-solid fraction. A TLC (1:1 hexane-diethyl ether mobile phase) of this neutral fraction showed four distinct and bright colored spots with the antimony trichloride spray. In order of mobility from fastest to slowest, the spots were red (monohydroxy triterpene), blue (sterol), red (triterpene aldehyde), and blue (dihydroxy triterpene). The neutral fraction was next chromatographed in hexane on silica gel (500 g) and each fraction (500 ml) was examined by TLC (with different ratios of hexane-diethyl ether). The combined fractions (2·7 g) eluted using hexane to 3 l. of 9:1 benzene–chloroform exhibited a camphor-like odor, negative LB test, and were not further studied. The next 4 l. of benzene–chloroform (9:1) eluted 1·44g of yellow oil (*fraction a*) which contained mainly the monohydroxy triterpenes. Elution with the final 2 l. of 9:1 benzene–chloroform to chloroform (2 l.) yielded 1·15 g of a yellow semi-solid (*fraction b*), which contained a sterol (0·18 g, m.p. 139–140·5° after chromatographic purification on a column of neutral alumina and recrystallization from methanol) closely related to the sitosterols and was not further characterized. Mixtures (1·6 g) of the sterol, the triterpene aldehydes (*fraction c*), and the dihydroxy triterpenes were eluted by the following: 3·5 l. of chloroform to the first liter of 95:5 chloroform–methanol. The next liter of chloroform–methanol (95:5) eluted 6·5 g of dark brown solid containing predominantly the dihydroxy triterpenes (*fraction d*).

[16] C. LIEBERMANN, *Ber. Deut. Chem. Ges.* **18**, 1803 (1885) and H. BURCHARD, *Ber. Deut. Chem. Ges.* **23R**, 752 (1890).

Further purification of the triterpene-containing fractions is described below in their respective sections.

Isolation of α-amyrin (IIb)

The 9:1 benzene–chloroform (*fraction a*) was dissolved in 1:1 benzene–hexane and rechromatographed on neutral alumina (45 g). Elution (100 ml fractions) with 19:1 benzene–chloroform yielded 0·30 g of colorless to yellow oils with aromatic odors. The fractions (0·70 g) eluted by 19:1 to 9:1 benzene–chloroform contained α-amyrin. Recrystallization from methanol led to needles melting at 160–170°. Three more recrystallizations from the same solvent gave a specimen of α-amyrin melting at 185–186°; $[\alpha]_D^{20}+73{\cdot}9°$ (*c*, 0·27 in methanol); mass spec. *m/e* 426 (M^+), 411, 408, 343, 218, 203, 189 and 133. Found: C, 84·27; H, 11·99; O, 3·74. $C_{30}H_{50}O$ (426) required: C, 84·44; H, 11·81; O, 3·75%.

The specimen of α-amyrin (IIb) showed a lemon-yellow color with tetranitromethane, a violet-red LB reaction and displayed one dark red spot on TLC (1:1 hexane–ethyl ether) sprayed with $SbCl_3$. Acetylation (5:3 pyridine-acetic anhydride, room temperature, 24 hr) yielded *α-amyrin acetate* melting at 205–215°. Four recrystallizations from methanol raised the melting point to 217–222°; $[\alpha]_D^{20}+76{\cdot}3°$ (*c*, 0·25 in chloroform).

A very pure sample of α-amyrin acetate was prepared as follows. The final two mother liquors obtained from recrystallizing the preceding sample of α-amyrin were concentrated. The crystalline second crop (0·10 g, m.p. 176–179°) was acetylated (*vide supra*) and the product crystallized from methanol to yield 0·08 g of acetate derivative (m.p. 198–210°) displaying one spot on TLC (85:15 hexane–diethyl ether mobile phase). A solution of the acetate in warm glacial acetic acid (8 ml) was treated with freshly sublimed SeO_2 (0·08 g). Before dilution with water and extraction with diethyl ether, the solution was heated at reflux 2 hr. Removal of solvent from the combined ethereal extract left a yellow vitreous residue which was chromatographed in 1:1 hexane–benzene on neutral alumina (3 g). Elution with the same solvent gave 0·064 g of colorless crystals melting from 210–224° (sintering from 203°). The product gave a deep yellow color with tetranitromethane and showed one spot on TLC (85:15 hexane–ethyl ether mobile phase). However, a $AgNO_3$ TLC employing the same solvent system showed two spots on development with sulfuric acid. The mono-olefin-diene mixture was separated by a preparative $AgNO_3$ TLC employing the 85:15 hexane–diethyl ether solvent system. By this means, 48 mg of *α-amyrin acetate* and 12 mg of impure diene were isolated. Recrystallizing the α-amyrin acetate from methanol yielded colorless plates melting at 224–226°; $[\alpha]_D^{20}+78{\cdot}8°$ (*c*, 0·24 in chloroform). The α-amyrin acetate was identical* with an authentic sample provided by Professor E. J. Corey.

The diene was further purified by repeating the $AgNO_3$ chromatographic procedure to yield 7 mg of colorless crystals melting at 165–195°, which gave an orange color with tetranitromethane and exhibited λ_{max} 243, 251 and 260 mμ (log ε 4·35, 4·41 and 4·22): final purification and characterization did not appear necessary.

Isolation of the Dihydroxy Triterpene

Recrystallizing the 19:1 chloroform–methanol fraction (*d*, 6·5 g, from the partially separated extract, see above) from methanol afforded 1·2 g of yellow solid which was chromatographed on neutral alumina (40 g). The first 150-ml portion of benzene–chloroform (1:1) eluted 0·16 g of a mixture containing the monohydroxy triterpenes, the sterol, and the

* The identical nature of both specimens was established by mixture melting point determination and i.r. spectral comparison in potassium bromide.

triterpene aldehydes. Continued elution with 200 ml of the same solvent led to 0·60 g of the *dihydroxy triterpenes*. Three recrystallizations from methanol gave colorless needles; m.p. 213–215°; $[\alpha]_D^{20}+40{\cdot}4°$ (*c*, 0·94 in methanol); mass spec. *m/e* 442 (M^+), 427, 424, 411, 409, 399, 393, 385, 381, 288, 286, 273, 271, 257, 247, 234, 216, 207, and 203 (the remaining fragmentation pattern was qualitatively similar to that of the triterpene aldehyde: see below). Found: C, 81·06, 81·02; H, 11·36, 11·66; O, 7·35, 7·34; active H, 0·39; mol. wt. (Rast) 436. $C_{30}H_{50}O_2$ (442) required: C, 81·39; H, 11·38; O, 7·23; active H, 0·46%.

The dihydroxy triterpenes showed one spot (bright blue with $SbCl_3$) on TLC (3:7 hexane–diethyl ether mobile phase) and gave a pale yellow color with tetranitromethane and a red-violet coloration in the LB test. Acetylation (2:3 acetic anhydride–pyridine, room temperature, 24 hr) of the diol (0·20 g) gave an acetyl derivative ($\lambda_{max}^{CHCl_3}$ 1735 and 1250 cm^{-1}) melting from 87–97°. Although the acetate displayed a single spot on TLC (7:3 hexane–diethyl ether), the wide melting point range and crystalline form was not improved by several recrystallizations using different solvents. This observation, combined with results of a SeO_2 oxidation study, indicated that the triterpene diol and its diacetyl derivative did not represent pure substances. SeO_2 oxidation of the acetate was accomplished as illustrated for purification of α-amyrin. The product displayed one spot on TLC but was shown to be a mixture of two components by TLC with the $AgNO_3$ technique (7:3 hexane–diethyl ether mobile phase). Preparative TLC of the reaction product (0·10 g from 0·15 g of the starting acetate) on a $AgNO_3$-treated plate gave 64 mg of the less polar product which still contained traces of diene (as evidenced by a $AgNO_3$ TLC Attempts at further purification and characterization were unsuccessful. The diene (5 mg, colorless crystals, λ_{max} 243, 251 and 260 mμ with log(4·14, 4·15 and 3·98, orange color with tetranitromethane) was also impure (m.p. 160–180°) but did suggest the presence of a β-amyrin-type compound.

Isolation of the Triterpene Aldehydes

Rechromatography of the chloroform to 19:1 chloroform–methanol fractions (*c*, 1·6 g, see initial separation of the hexane extract) in benzene on neutral alumina (50 g) gave in the 19:1 benzene–chloroform fractions 0·53 g of yellow oil containing a crude triterpene aldehyde ($\nu_{max}^{CHCl_3}$ 1725 cm^{-1}). TLC indicated that the aldehyde was accompanied by several impurities and for further purification the crude mixture was acetylated (see above) and the *acetoxy aldehyde* (viscous yellow oil) was crystallized from hexane (3 times) and methanol to afford colorless leaflets (0·078 g) decomposing at 223–236°; ν_{max} 2900, 1728, 1710, 1450, 1370, 1250, 1150, 1095, 1025, 1000, and 970 cm^{-1}; mass spec. *m/e* 482 (M^+), 467, 464, 453, 452, 439, 422, 407, 394, 393, 382, 379, 351, 284, 271, 269, 257, 249, 232, 203, 190, 189, 175, 161, 147, 133, 121, 119, 109, 107, 105, 95, 93, 91, 81, 69, 55, 43, and 41. Found: C, 79·89; H, 10·49; O, 9·62. $C_{32}H_{50}O_3$ (482) required: C, 79·62; H, 10·44; O, 9·94%.

The acetate derivative gave a lemon yellow color with tetranitromethane and a deep violet LB reaction. Although an isomeric mixture, TLC (3:2 hexane–diethyl ether mobile phase) of the acetate showed only a single spot.

Reduction of the acetate derivative (20 mg) in diethyl ether (5 ml) with excess lithium aluminum hydride during 1 hr gave, after crystallization from methanol, 5 mg of a diol melting at 224–228° (sintering from 215°). The hydroxy derivative was identical* with the triterpene diols described in the preceding experiment. A mixture of both diol samples melted at 217–220° and exhibited the same polarity and deep blue development ($SbCl_3$ spray) color on TLC (3:7 hexane–diethyl ether mobile phase).

* See footnote on p. 306.

Isolation of Oleanolic and Ursolic Acids

A sample (10 g) of the solid material (53 g) which separated during concentration of the original diethyl ether extract (see extraction procedure) was selected for purification studies. TLC (75:21:4 carbon tetrachloride–acetone–methanol mobile phase)[17] gave evidence of only one component in quantity with the same mobility and orange-red fluorescence in u.v. light ($SbCl_3$ spray) as authentic ursolic acid. Dissolution of the solid (10 g) in ethanol (300 ml) by warming gave, upon cooling, a gelatinous material which was collected and dissolved in excess ethanol. The resulting solution was filtered to remove a small amount of brown flocculent material. The ethanol solution was reduced in volume, cooled and the gelatinous phase was again separated. As the fractional recrystallization process continued, the gelatinous phase became more crystalline and after repeating the process nine more times crystals melting at 278–280° were obtained. Three additional recrystallizations yielded a pure specimen (0·20 g) of ursolic acid, m.p. 281–283°. The acid was identical* with an authentic specimen of *ursolic acid* kindly provided by Prof. Brieskorn.[6]

A sample (50 mg) of ursolic acid was acetylated (see α-amyrin acetate) and the product recrystallized from ethanol to yield *3β-acetyl-ursolic acid* (30 mg) melting at 285–289°, $[\alpha]_D^{20}+53°$ (*c*, 0·45 in chloroform). Treating the acetyl derivative with diazomethane† and recrystallizing the product from ethanol gave *3β-acetyl-ursolic acid methyl ester* (20 mg) melting at 243–246°. Recrystallizing the methyl ester from methanol–ethyl acetate yielded an analytical specimen as needles melting at 246–247°; $[\alpha]_D^{22}+70·4°$ (*c*, 0·66 in chloroform). The methyl ester was identical* with an authentic specimen and was further supported by results of elemental analyses. Found: C, 77·36; H, 9·87. $C_{33}H_{52}O_4$ required: C, 77·30; H, 10·22%.

Partial saponification (5% KOH in methanol, 30 min at reflux) of 3β-acetyl ursolic methyl ester yielded *ursolic acid methyl ester*. Recrystallization from petroleum ether (b.p. 30–60°) gave needles melting at 109–111° with resolidification and final melting at 166–168°; $[\alpha]_D^{22}+70·9°$ (*c*, 0·74 in chloroform). Found: C, 78·92; H, 10·74. $C_{31}H_{50}O_3$ required: C, 79·10; H, 10·71%.

In order to isolate oleanolic acid, the first mother liquor from the ursolic acid recrystallization sequence was slowly concentrated and the third crystal crop was recrystallized from ethanol. Again, the mother liquor solution was slowly concentrated and the sixth crystalline crop was recrystallized from ethanol to yield *oleanolic acid* (40 mg), m.p. 298–302°, identical* with an authentic commercial (Penick) specimen. The 3β-acetyl derivative (see 3β-acetyl-α-amyrin) crystallized from ethanol as needles melting at 258–262° and was also identical* with an authentic sample.

Isolation of Ursolic Acid from Salvia karwinskii

Salvia karwinskii is a herbaceous plant 2–3 ft in height with scarlet flowers and the complete (stems, leaves and flowers) plant, air-dried and ground, was used in the present study. The specimen employed was collected near San Jose, east of the main highway to San Cristobal, Chiapas, Mexico, by Dr. B. G. Schubert and A. Gomez Pompa in March, 1958. Extraction was accomplished by stirring the plant material (855 g) in refluxing light petrol (30–60° 2 × 3 l.) for 24 hr. After drying, the process was repeated with 2 × 3 l. of diethyl ether.

[17] H. KLINGER, *Die Triterpene und Sterine des Fruchtschale und des Blattes von* Pinus Malus *L.*, Dissertation Universität Würzburg (1962).

* See footnote on p. 306.

† We wish to thank Dr. U. R. Ghatak for performing this experiment.

Following concentration, the light petrol extract yielded 4·2 g and the diethyl ether extract 7·7 g of residue. Using a soxhlet technique and 350 g of *S. karwinskii*, the respective yield of petroleum ether, diethyl ether and 95% ethyl alcohol extracts were 2·7, 2·1 and 20·5 g. The former 4·2-g light petrol extract was treated with 200 ml of diethyl ether. All but 1·8 g of a light green powder dissolved and *ursolic acid* was eventually isolated from this fraction as described in detail for *Salvia apiana*. The triterpene acid and its acetate and methyl ester derivatives were identical* with authentic specimens.

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* See footnote on p. 306.