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SESQUITERPENE LACTONES OF *ARTEMISIA* SPECIES. NEW LACTONES FROM *A. ARBUSCULA* SSP. *ARBUSCULA* AND *A. TRIPARTITA* SSP. *RUPICOLA*

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Abstract—The new santanolides, arbusculin-A, -B and -E, have been isolated from *Artemisia arbuscula* Nutt. ssp. *arbuscula*: and the closely related colartin (11,13-dihydroarbusculin-A) has been found in *A. tripartita* Gray ssp. *rupicola* Beetle. These four lactones appear to represent early stages near the origin of the biosynthetic pathway of formation of compounds of this class.

INTRODUCTION

THE SECTION Seriphidium of the genus *Artemisia* includes a group of species that have been classed as the section Tridentatae Rydb.[2] This section, many members of which fall under the popular rubric of "Western Sagebrush", has been under systematic examination in this laboratory,[3,4] and is the subject of continuing investigation. Studies of *A. arbuscula* Nutt. ssp. *arbuscula*, and of a new collection of *A. tripartita* Gray ssp. *rupicola* Beetle have disclosed the presence of a number of new lactones, closely related in structure and suggestive of a sequence of biosynthetic transformations. Six new compounds have been isolated, of which the four described here were obtained in sufficient amount to permit establishment of their structures. Two additional lactones are still under study.

RESULTS AND DISCUSSION

Four separate collections[5] of *Artemisia arbuscula* ssp. *arbuscula* were found to be essentially identical by TLC examination of their extracts.

Arbusculin-A (I) (Chart 1), $C_{15}H_{22}O_3$, m.p. 76·5–77·5°, showed i.r. absorption at 3556 (OH), 1768 (γ-lactone), and 1663 (α-methylene) cm^{-1}, and prominent mass spectral peaks at *m/e* 250 (M^+), M-15, M-18 and M-15–18. The 6/7 position and stereochemistry of the lactone ring was manifest in the NMR spectrum (Table 1), in which the C*H*—O proton at C-6 was seen as a triplet with the coupling constants of 11 Hz.

[1] Contribution No. 2419 from the Department of Chemistry, U.C.L.A.

[2] A. A. BEETLE, *Rhodora* **61**, 82 (1959).

[3] T. A. GEISSMAN, T. STEWART and M. A. IRWIN, *Phytochem.* **6**, 901 (1967).

[4] M. A. IRWIN and T. A. GEISSMAN, *Phytochem.* **8**, 305 (1969).

[5] We are indebted to Professors A. A. Beetle and R. O. Asplund (University of Wyoming) and G. H. Ward (Knox College) for collecting and identifying the specimens used in this work. Collections identified by voucher numbers AAB-966-AAA, ROA-90767-AAAR, GHW-966-AAT and AAB-966-AAT were used in this work. It is to be noted that a collection called *A. tripartita rupicola* was essentially identical (TLC) with the above materials and quite dissimilar to earlier collections of *A. tri. rup.*, an indication of the taxonomic difficulties associated with some members of the section.

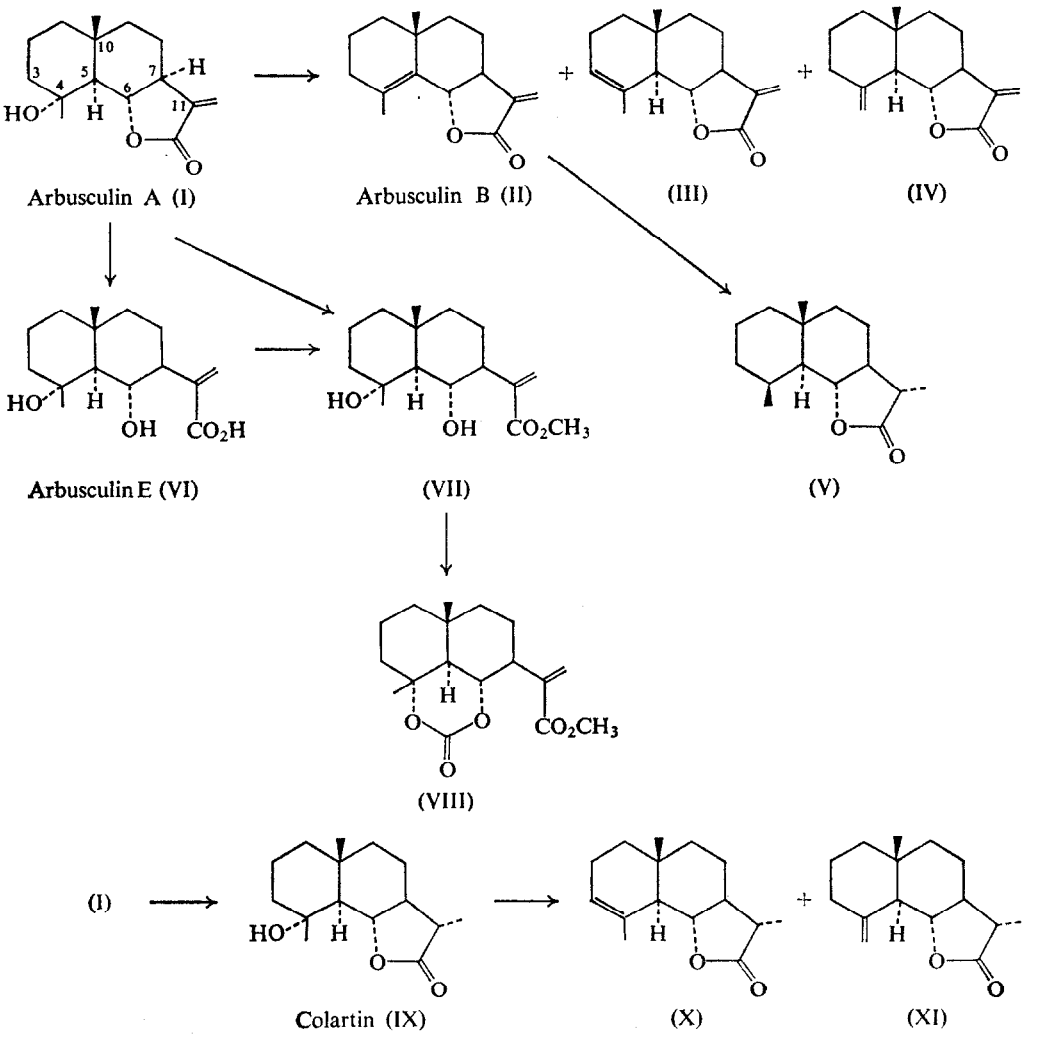

CHART 1.

Treatment of arbusculin-A with thionyl chloride–pyridine yielded a mixture of the three double-bond isomers, II–IV. Of these, the minor product (II) was isolated in crystalline form and proved to be identical with arbusculin-B, 86.5–88°, a constituent of the plant. Arbusculin-B (II) had i.r. absorption at 1762 (γ-lactone) and 1663 (α-methylene)cm^{-1} and the expected mass spectral peaks at *m/e* 232 (M^+) and M-15. Its NMR spectrum showed the lactonic proton (C*H*—O) as a doublet ($J = 11{\cdot}5$ Hz, coupling with H at C-7), each component of which was further split ($J = 1{\cdot}5$ Hz) by homallylic coupling to the methyl group at C-4. The latter (δ 1·86) appeared as a doublet with the appropriate coupling constant ($J = ca.$ 1 Hz).

Hydrogenation of arbusculin-B yielded santanolide-C (V), identical (mixed m.p., i.r.) with a specimen derived from vulgarin.[6]

[6] T. A. GEISSMAN and G. A. ELLESTAD, *J. Org. Chem.* **27**, 1855 (1962).

TABLE 1. NMR SPECTRA OF *Artemisia* SESQUITERPENE LACTONES (60 MHz)

Cpd.	C-3	C-4	C-5	C-6	C-7	C-10	C-11
I		CH_3: 1·32 OH: 2·99	H: 1·81 (11·5-d)	H: 4·05 (11-d, 11·5-d)	H: 2·58 (11-t, 3-t)†	CH_3: 0·99	CH_2: 6·07 (3-d), 5·46 (3-d)
II		CH_3: 1·86 (*c.* 1-d)	—	H: 4·52 (11·5-d, 1·5-q)	H: 2·53 (11-t, 3-t)†	CH_3: 1·11	CH_2: 6·09 (3-d), 5·52 (3-d)
III	H: *c.* 5·35 (br)	CH_3: *c.* 1·84 (br)		H: 3·84 (11-d, 10-d)		CH_3: 0·90	CH_2: 6·00 (3-d), 5·38 (3-d)
IV		CH_2: 4·90 (br), 4·77 (br)		H: 3·93 (10·5-t)		CH_3: 0·83	CH_2: 6·00 (3-d), 5·38 (3-d)
V		CH_3: 1·01 (7·5-d)		H: 3·94 (11-d, 9-d)*		CH_3: 1·04	CH_3: 1·19 (6·5-d)
VII		CH_3: 1·32 OH: *c.* 3·8 (br)	H: 1·43 (*c.* 10-d)	H: 4·08 (10-d, 10·5-d) OH: *c.* 3·8 (br)		CH_3: 0·96	CH_2: 6·23 (1-d), 5·69 (1-t) CO_2CH_3: 3·75
VIII		CH_3: 1·47	H: 1·58 (*c.* 12-d)	H: 4·82 (10-d, 12-d)	H: 2·64 (m)	CH_3: 1·02	CH_2: 6·28 (1-d), 5·71 (1-t) CO_2CH_3: 3·75
IX		CH_3: 1·31 OH: 3·05	H: 1·67 (11·5-d)	H: 4·08 (11·5-d, 9·5-d)*		CH_3: 0·99	CH_3: 1·19 (6·5-d)
X	H: 5·39 (br)	CH_3: 1·82 (br)		H: 3·89 (*c.* 10-t)*		CH_3: 0·93	CH_3: 1·22 (6·5-d)
XI		CH_2: 4·94 (br), 4·78 (br)		H: 3·99 (*c.* 10-t)*		CH_3: 0·86	CH_3: 1·21 (6·5-d)

* Irregular.

† Plus additional coupling to C-8 H.

Spectra measured in duterochloroform with tetramethylsilane as internal standard and chemical shifts reported in δ units and couplings (J) in Hz.

Arbusculin-E, (VI), $C_{15}H_{24}O_4$, m.p. 160–161°, isolated from the plant extracts, was found to be the acid derived by opening of the lactone ring of arbusculin-A. There is some, but inconclusive evidence, that it is present in the original extracts of the plant; the compound is found on TLC plates in a region obscured by other components of the crude mixture, and is present in small amounts only. It is possible that it is an artefact, derived by adventitious ring opening of the lactone I during the isolation process, although the conditions used in processing the crude extracts do not, in our experience, appear to be sufficient for this ring opening. The acid VI was further characterized by the preparation of its methyl ester (VII).

Further evidence for the stereochemical relationships shown in I, II and VI is found in the preparation of the cyclic carbonate VIII by treatment of the ester VII with phosgene–pyridine. This observation, coupled with the preferential dehydration of I to III and IV, established the α-configuration of the C-4 hydroxyl group and thus the complete stereochemistry shown in the structures assigned to these compounds.

Further examination of a collection of *A. tripartita* ssp. *rupicola* which has been described as containing cumambrins A and B, deoxycumambrin,[4] has disclosed the presence of two new lactones (still under study), ATR-3 and ATR-14, and a compound, colartin, that proved to be 11,13-dihydroarbusculin-A. Colartin (IX), $C_{15}H_{24}O_3$, m.p. 107–108°, showed i.r. absorption at 3509 (OH), 1778 (γ-lactone) cm^{-1} and prominent peaks in the mass spectrum at *m/e* 252 (M^+), M-15, M-18 and M-15–18. Its NMR spectrum showed the absence of the α-methylene grouping of the lactone and the presence of a signal for the C-11 methyl group of the saturated lactone (δ 1·19, 3H, d, $J = 6·5$ Hz). The structure of colartin was established by its preparation from arbusculin-A by catalytic hydrogenation, a procedure that provides the α-disposed C-11 methyl group (cf. the conversion of II into V). The dehydration of colartin with sulfuric acid-acetic anhydride produced the isomeric unsaturated compounds X and XI, known from earlier work[7] as α- and β-cyclodihydrocostunolide. The appearance of the double bond in the 3,4- and the exo positions is in accord with the α-disposition of the C-4 hydroxyl group.

[7] A. M. SHALIGRAM, A. S. RAO and S. C. BHATTACHARYA, *Tetrahedron* **18**, 969 (1962).

The structures of I, II, VI and IX are related in a simple manner to that of costunolide (XII), for their derivation from XII by a straightforward acid-catalyzed ring closure, as shown in Chart 2, is unexceptional. The point at which colartin is formed by saturation of the 11,13-double bond cannot be inferred from these suppositions. It is to be noted that costunolide, known in other genera of the Compositae, has been found in *A. balchanorum*.[8]

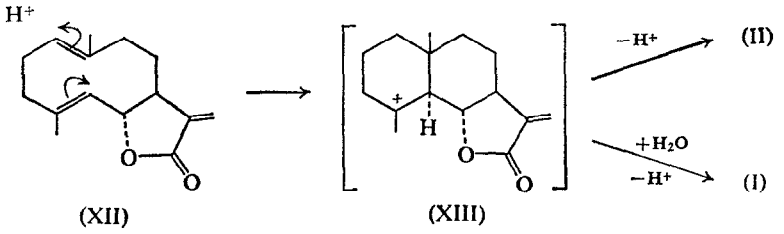

CHART 2.

EXPERIMENTAL

TLC was done with silica gel G-coated plates developed in acetone–$CHCl_3$ and stained with H_2SO_4 spray. The spot color that appeared for all of the compounds reported was gray to blue-gray. M.ps were taken in capillaries and are corrected. Spectral measurements were made with Perkin-Elmer 237 (i.r. in $CHCl_3$), AEI MS-9 (m.s., 70 eV, direct insertion method) and Varian A-60 and A-60D (NMR) instruments. NMR data are given in Table 1.

Isolation of Arbusculin A, B, C, D and E

Dry, milled *Artemisia arbuscula arbuscula* (1·3 kg, voucher No. AAB-966-AAA) was extracted with 3 × 2 l. of $CHCl_3$, the concentrated extract was partitioned between 1·5 l. of hexane and 1·5 l. of methanol–water (3:1). Each phase was extracted with 1 l. of fresh solvent and the 1 l. phases were extracted with each other. The hexane extract contained the greater fraction of arbusculin-B as the only compound in common with the methanol–water extract (TLC). The total hexane extract was evaporated and chromatographed over a column (6 × 38 cm) of silica gel eluted with pentane–$CHCl_3$ (1:1). The fractions containing arbusculin-B (AAA-2) were combined and evaporated to a crystalline residue from which a wax crystallized and was removed. The total methanol–water extract was evaporated on a hot-water bath under reduced pressure. The residue was chromatographed over a column (8 × 46 cm) of silica gel with thirty-four 0·5-l. fractions being eluted with $CHCl_3$ benzene and gradually increasing proportions of acetone in $CHCl_3$–benzene and $CHCl_3$ to acetone and evaporated.

Fraction 1 and the arbusculin-B-containing fraction from the hexane extract were combined and chromatographed over silica gel, eluted with $CHCl_3$–benzene (1:1). The arbusculin-B-containing fractions were combined and evaporated to a residue which yielded 740 mg (0·057 per cent yield) of crystals. Recrystallized from ether–petroleum ether as needles, the analytical sample had m.p. 86·5–88°; $[\alpha]_D^{24}$ +47·3° (*c*. 2·77, $CHCl_3$); circular dichroism $[\theta]_{249}$ −4040 (methanol); i.r. spectrum 1762 (s) and 1663 cm^{-1} (w); mass spectrum *m/e* 232 (M^+) and 217 (base). (Found: C, 77·59; H, 8·57. Calc. for $C_{15}H_{20}O_2$: C, 77·55; H, 8·68.) It was identical (m.m.p., i.r.) to anhydroarbusculin-A (II).

Fraction 2 yielded 620 mg (0·048 per cent yield) of arbusculin-C (AAA-1).

Fractions 3–5, containing arbusculin-A (AAA-3), tended to be oily at room temperature and were treated with activated charcoal and crystallized from petroleum ether in a dry ice–hexane bath giving 1800 mg (0·14 per cent yield) of crystals. Recrystallized from ether–petroleum ether as plates, the analytical sample had m.p. 76·5–77·5°; $[\alpha]_D^{24}$ +25·8° (*c*. 4·15, $CHCl_3$); i.r. spectrum 3556 (m), 3390 (w, broad), 1768 (s) and 1663 cm^{-1} (w); mass spectrum *m/e* 250 (M^+), 245 (base), 243, 217, 204 and 189. (Found: C, 72·19; H, 8·76. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86.)

Fractions 16–20 contained non-crystalline arbusculin-D (AAA-4).

Arbusculin-E (AAA-5), contained in fractions 25–28, was extracted from 0·25 l. $CHCl_3$ into 0·3 l. of 0·7 M Na_2CO_3. The acidified aqueous phase was extracted with 3 × 0·5 l. $CHCl_3$ and the extract evaporated to a non-crystalline residue (0·1 g of arbusculin-E; 0·008 per cent yield). Part of it was chromatographed (silica gel, acetone–$CHCl_3$–benzene) and the concentrated arbusculin-E-containing fractions after several days yielded crystals (triturated with ether, m.p. 159–160°) which were identical (m.m.p., i.r.) with hydrolyzed arbusculin-A.

[8] V. HEROUT, M. SUCHY and F. SORM, *Coll. Czech. Chem. Commun.* **26**, 2612 (1961).

From fraction 9, from fraction 12 and from fractions 14–15 were obtained AAA-8, AAA-6 and AAA-7, respectively, crystalline flavonoids.

Isolation of Colartin

Dry, milled *A. tripartita rupicola* (0·36 kg, voucher No. GHW-966-ATR) was extracted with $CHCl_3$. The concentrated extract was mixed with 0·5 l. of water and steam was passed through the mixture until the vapor was 90°. After cooling, the aqueous phase was decanted, filtered through celite and extracted with 3 × 0·5 l. $CHCl_3$. The celite pad was returned to the tar, and this process was repeated on the tar five more times. The total $CHCl_3$ extract was evaporated to a gum (12 g) which was chromatographed over a column (5 cm) of silica gel (0·4 kg), eluted initially with $CHCl_3$. Fractions (0·4 l.) 3–4 contained colartin (ATR-9) which crystallized from ether–petroleum ether as plates, 0·4 g (0·12 per cent yield). Later fractions yielded cumambrin-A and -B, deoxycumambrin, coumarins and ATR-3 and -14. The colartin was purified by chromatography and a sample crystallized from petroleum ether was identical (m.m.p., i.r.) with dihydroarbusculin-A.

Arbusculin-B (Anhydroarbusculin-A, II)

Arbusculin-A (200 mg) was treated in 2 ml of pyridine with 0·5 ml $SOCl_2$. The solution was diluted with water and extracted with $CHCl_3$. The extract, freed of solvent, contained (NMR) II, III and IV in relative proportions of about 2:3:6. The mixture was carefully chromatographed (silica gel, $CHCl_3$) and the fractions containing II (determined by NMR) were crystallized from ether–petroleum ether giving material identical (m.m.p., i.r.) with the natural sample of arbusculin-B.

Tetrahydroarbusculin-B (Santanolide-C, V)

Arbusculin-B (260 mg) and 120 mg of 10% Pd–C in 15 ml of methanol presaturated with H_2 took up 65 ml (theory 57 ml) of H_2 overnight. The solution was filtered and the solute recrystallized twice from ethyl acetate–ether giving 60 mg of plates having m.p. 154–157°; $[\alpha]_D^{24}$ +47·0° (*c.* 5·44, $CHCl_3$); i.r. spectrum 1765 cm^{-1} (s). It was identical (m.m.p., i.r.) with santanolide-C prepared from vulgarin.[6]

Arbusculin-E (Hydrolyzed Arbusculin-A, VI)

Arbusculin-A (90 mg) in 0·5 ml of methanol was stirred with 110 mg KOH in 6 ml of water for 0·5 hr giving a clear solution. Acetic acid (1 ml) and 15 ml of water were added and the solution was extracted with 3 × 50 ml $CHCl_3$. The extract was dried, evaporated, treated with activated charcoal and crystallized from ether giving 30 mg of crystals identical (m.m.p., i.r.) with the natural sample of arbusculin-E. This sample had m.p. 160–161°; $[\alpha]_D^{24}$ +3° (*c.* 0·75, $CHCl_3$); mass spectrum (no M^+, 268) *m/e* 253, 250, 235, 232, 217, 204 and 189; i.r. spectrum 3569 (w), 3345 (m, broad), 2650 (m, broad), 1693 (s) and 1620 cm^{-1} (m). (Found: C, 67·34; H, 9·13. Calc. for $C_{15}H_{24}O_4$: C, 67·13; H, 9·02.)

Arbusculin-E Methyl Ester (VII)

Part of the oily arbusculin-E (0·05 g) in methanol was treated with excess CH_2N_2 in ether. After 2 hr the solution contained a single new compound (TLC) and it was evaporated and chromatographed (silica gel, acetone–$CHCl_3$–benzene). The compound-containing fractions were combined, evaporated, treated with activated charcoal and crystallized from ether–petroleum ether giving 5 mg of needles.

Arbusculin-A (170 mg) in 7 ml of methanol containing 5 drops of conc. HCl stood overnight. The reaction contained (TLC) a new low R_f compound and a small amount of starting material and higher R_f substances. The solution was evaporated and chromatographed (silica gel, acetone–$CHCl_3$–benzene). The compound-containing fractions were evaporated, treated with activated charcoal and crystallized from ether–petroleum ether giving 60 mg of needles identical (m.m.p., i.r.) with the above compound. This sample had m.p. 106–107°; i.r. spectrum 3575 (w), 3401 (m, broad), 1711 (s) and 1621 cm^{-1} (m); u.v. spectrum (ethanol), end absorption; mass spectrum (no M^+, 282), *m/e* 267, 264, 249, 235, 232 (base), 217, 204 and 189. (Found: C, 68·07; H, 9·03. Calc. for $C_{16}H_{26}O_4$: C, 68·05; H, 9·28.)

Arbusculin-E Carbonate Methyl Ester (VIII)

The mother liquor of the ester (VII) from its preparation from arbusculin-A above was evaporated (40 mg) and dissolved in 3 ml $CHCl_3$ containing 5 drops of pyridine. Phosgene was slowly passed into the solution for 2 min after which was seen a single compound of higher R_f. The solution was diluted with 100 ml of water and extracted with 2 × 100 ml $CHCl_3$. The extract was chromatographed (silica gel, $CHCl_3$–benzene, 1:1) and the compound-containing fractions evaporated, treated with activated charcoal and crystallized twice from ether–petroleum ether giving 24 mg of granules having m.p. 115·5–116°; i.r. spectrum 1721 (s, broad) and 1620 cm^{-1} (m); mass spectrum *m/e* 308 (M^+), 264, 249, 246, 232 and 217 (base). (Found: C, 66·52, 66·48; H, 7·84, 7·75. Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·85.)

Colartin (Dihydroarbusculin-A, IX)

Arbusculin-A (180 mg) and 150 mg of 10% Pd–C in 25 ml EtOAc was saturated with H_2 (0·3 hr). The solution contained two compounds of similar R_f and was filtered and chromatographed (silica gel, $CHCl_3$–

benzene, 1:1). The fractions containing the higher R_f compound were evaporated, treated with activated charcoal and crystallized from ether–petroleum ether giving 40 mg of plates. It was identical (m.m.p., i.r.) with natural colartin and had m.p. 107–108; $[\alpha]_D^{24}$ +11·4° (*c.* 3·67, $CHCl_3$); i.r. spectrum 3509 (m) and 1718 cm^{-1} (s); mass spectrum *m/e* 252 (M^+), 237 (base), 234, 219, 209, 206, and 191. (Found: C, 76·90; H, 9·53. Calc. for $C_{15}H_{24}O_3$: C, 71·39; H, 9·59.)

Anhydrocolartins (X, XI)

A solution of 60 mg of colartin in 1 ml Ac_2O containing a trace of H_2SO_4 was stirred a few minutes. Water (3 ml) was added, and the solution was stirred overnight and extracted with 3 × 3 ml of benzene. TLC showed two spots of higher R_f than that of colartin. The extract was chromatographed and fractions containing the slightly higher R_f compound, X, were combined, evaporated and sublimed (150°, vac.) and washed with petroleum ether giving 10 mg of plates. It contained (TLC) a trace of XI and had m.p. 132–135° (lit.[7] m.p. 140°); i.r. spectrum 1767 cm^{-1} (s); mass spectrum *m/e* 234 (M^+), 219 (base), 205, 204 and 191. (Found: C, 76·90; H, 9·53. Calc. for $C_{15}H_{22}O_2$: C, 76·88; H, 9·46.)

Fractions containing the slightly lower R_f compound were treated similarly giving 10 mg of plates containing (TLC) a trace of X and having m.p. 138·5–140° (lit.[7] m.p. 140°); i.r. spectrum 1764 cm^{-1} (s); mass spectrum *m/e* 234 (M^+), 219, 206 and 191. (Found: C, 76·78; H, 9·36. Calc. for $C_{15}H_{22}O_2$: C, 76·88; H, 9·46.)

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