# Search for New Industrial Oils. X. Seed Oils of the Calenduleae

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## Abstract

Seed oils from 29 species in five genera of the tribe Calenduleae, family Compositae, have been examined to determine the distribution of dimorphecolic acid (9-hydroxyl-*trans*-10,*trans*-12-octadecadienoic acid) among the close relatives of *Dimorphotheca*. Dimorphecolic acid occurs in all five of the *Dimorphotheca* species analyzed, in *Castalis nudicaulis*, and in five species of *Osteospermum* in amounts ranging from 34–75% of the oil. In all other species of the tribe analyzed to date, including 14 species of *Osteospermum*, two of *Calendula*, and two of *Chrysanthemoides*, the oil contained conjugated trienoic acids ranging from 14–60%.

## Introduction

THE UNUSUAL combination of functional groups in dimorphecolic acid (9-hydroxy-*trans*-10,*trans*-12-octadecadienoic acid), first isolated and characterized at the No. Reg. Res. Lab. (5,15), should provide opportunities for the preparation of novel chemical derivatives not readily obtainable from other seed oils. Particularly attractive from a utilization standpoint is the occurrence of dimorphecolic acid in high concentrations (over 60%) in some of the oils investigated. Accordingly, a search was begun for the best plant source of this recently discovered acid. This report presents various chemical data on seeds obtained by USDA botanists, mainly from wild stands in southern Africa. During the collection special attention was given to plant groups closely related to the genus *Dimorphotheca* in the tribe Calenduleae, family Compositae.

### Botanical Aspects of the Tribe Calenduleae

The tribe Calenduleae as interpreted by Norlindh (13) includes nine genera, eight of which are restricted to the Old World. There the tribe has two main centers of distribution: the Mediterranean region and southern Africa. In southern Africa, where the tribe has attained its greatest differentiation into genera and species, it is represented by *Dimorphotheca, Castalis, Osteospermum, Chrysanthemoides, Gibbaria,* and *Garuleum.* In the Mediterranean center, only *Calendula* and *Dipterocome* occur. The only representative of the tribe in the New World is *Eriachaenium,* which occurs in southern Chile (14). *Osteospermum* is by far the largest genus containing 67 species as delineated by Norlindh in 1943. *Calendula,* containing about 15 species (16), is second. The remaining genera are still smaller, and *Dipterocome* and *Eriachaenium* contain but a single species each (13).

The members of the tribe comprise a heterogeneous assemblage of plants so far as habit is concerned and, aside from the structures associated with reproduction, few generalizations can be made for the group as a whole. The members may be diminutive annuals or arborescent shrubs, and nearly every aspect between these extremes is represented. Fruit productivity varies considerably, and the fruits themselves may range from light, membranous-winged achenes to bony-hulled, nutlike structures to drupes.

The extensive natural range occupied by members of the Calenduleae in southern Africa and the Mediterranean region encompasses a wide diversity of climatic and edaphic conditions, many of which can be closely approximated in different areas of the U.S. A number of species in the Calenduleae have been cultivated as ornamentals for years, but the only species widely planted in the U.S. are *Calendula officinalis* (pot marigold) and *Dimorphotheca sinuata* (cape marigold).

A botanical survey of the wild populations in southern Africa revealed a sizable group of species with a wide spectrum of variation in characteristics. No species is presently suitable for modern methods of cultivation and harvest and for producing large yields of seed. However, the variability within this plexus of favorable species suggests eventual success for research to develop suitable lines.

## Methods

Sample handling depended on the physical structure of the seed (or fruit) and on the amount available. If easily removable, pericarp was taken off and its percentage determined by weighing. Samples available in adequate quantities were ground through a screen with $\frac{1}{16}$-in. round holes in a 6-in. hammer mill, and 3-g samples were used for oil analysis. The samples were extracted for 6 hr with petroleum ether in a Butt apparatus. Nitrogen content was determined by the Kjeldahl procedure, and moistures were determined by ovendrying at 130C. When the sample was too small for such procedures, crude protein determination was omitted, moisture content was assumed on the basis of related meals handled similarly, and selected analyses were made on the oil available. Some of the smallest samples were ground in an intermediate Wiley mill.

Hydrogen bromide titration was carried out as recommended for the determination of oxirane oxygen (AOCS Tentative Method Cd 9–57). Spectrophotometric measurements in the UV were made on the oils dissolved in ethanol, essentially as described for the measurement of preformed conjugation in the AOCS method Cd 7-58 for polyunsaturated acids. Percentage of dimorphecolic acid was calculated from the absorption at 233 m$\mu$, using a molecular extinction coefficient $\epsilon$ of 33,500 (6). Conjugated trienes were calculated as $\alpha$-eleostearic acid from the absorption of the peak at 270–272 m$\mu$, using $\epsilon = 47{,}000$ (2). Diene was not measured in samples rich in triene because of overlapping absorption.

GLC analyses were carried out according to the procedure of Miwa et al. (10). The percentage of conjugated trienoic methyl esters was calculated from the total area under multiple peaks (equivalent chain lengths, 19.2–19.7, Apiezon L) attributed to their various geometric isomers. The figures reported include dehydration products from the small amounts of dimorphecolic acid present in the oils, but do not include any eicosenoic acid, as evidenced by analysis on a Resoflex 446 column.

## Results

Examination of Table I reveals that the seed oils from every one of the 88 samples analyzed, representing 29 species in five genera, contained either di-

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morphecolic acid or conjugated trienoic acid as a major constituent.

Dimorphecolic acid is the characteristic component in all reported samples of *Dimorphotheca* (five species) and *Castalis* (one species). It is also the principal acid of five species of *Osteospermum: O. caulescens, O. dregei, O. ecklonis, O. fruticosum,* and *O. jucundum.* Its occurrence in these species is consistent with taxonomic classification based on morphology since the five *Osteospermum* species mentioned constitute section *Blaxium* of the genus, and this section together with *Castalis* comprises the members of the tribe Calenduleae most closely related to *Dimorphotheca.*

When large quantities of dimorphecolic acid were present, agreement was usually good between the amount determined by UV absorption and that by hydrogen bromide titration, provided that the latter determination was carried out rapidly. Otherwise, the amount determined by titration was low, possibly due at least in part to reactions noted earlier (8) in which hydrogen bromide appears to react with dimorphecolic acid to give a *trans* diene, which then eliminates a molecule of hydrogen bromide to react again. We have chosen to report in Table I only those values for dimorphecolic acid content obtained by UV analysis except when the presence of major amounts of triene obscured the diene absorption, in which case hydrogen bromide titration data are provided.

Among the species not containing conjugated diene in large percentage, the average amounts of conjugated triene varied from 16–54%. Highest concentration was in the genus *Calendula,* samples of which included different cultivated varieties of *C. officinalis* and a sample of *C. arvensis* that was obtained from Pakistan. The lowest amount of conjugated triene in this group was 14% in one sample of *Osteospermum imbricatum.*

GLC analyses were carried out on several samples of the oils containing large percentages of conjugated triene. The agreement in Table II between GLC and UV analyses for the conjugated trienoid acids, while not as close as may be desired, confirms the relative amounts present in the various species. The disparity might be reduced by determination of the conjugated trienoic isomer present in each species, by careful calibration of GLC detector response to conjugated trienoic standards, and by using the UV absorption coefficient for the appropriate isomer. GLC analysis was not reported on the oils containing large amounts of dimorphecolic acid because of the sensitivity of this acid to dehydration (1).

For two samples of *Dimorphotheca pluvialis* and for *D. chrysanthemifolia* it was possible to analyze separately seed from the (outer) ray florets and (inner) disk florets, which differ in appearance and impart apparent heterogeneity to the sample. However, the percentages of oil, protein, and pericarp, and the fatty acid compositions did not differ for the two types of seed of the two species tested.

## Discussion

Seed oils from many plant genera show presumptive evidence of the presence of dimorphecolic acid or related compounds in small amounts (3–5). They absorb UV light in the region expected for conjugated dienes and react with hydrogen bromide under conditions of the oxirane oxygen determination. These genera include about two-fifths of the representatives of the family Compositae tested up to this time and smaller proportions from a number of other families. Morris et al. (11) suggested on the basis of TLC that several species of composites contain, besides

TABLE I

Data on Calenduleae Seed and Oils[a]

| Source | Pericarp removed, % | Component analyzed[b] | Component analyses | | | Oil analyses | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Wt/1,000,[c] g | Oil, dry basis, % | Protein (NX6.25), dry basis % | Iodine value, Wijs | $n_D^{40}$ | Apparent dimorphecolic acid,[d] % | Conj. triene by UV, % |
| *Calendula arvensis* L. | 71 | S | 2.6 | 41 | 41 | ..... | ..... | 7(A) | 54 |
| *Calendula officinalis* L. | 64(19) | S | 3.1(20) | 44(20) | 33(19) | 154 | 1.4964 | 4(19)(A) | 51(18) |
| *Castalis nudicaulis* DC. | ..... | S + P | ..... | 11[e] | ..... | ..... | ..... | 34(B) | 4 |
| *Castalis nudicaulis* var. *graminifolia* (L.) T. Norl. | ..... | S + P | ..... | 8[e] | ..... | ..... | ..... | 48(B) | 6 |
| *Chrysanthemoides incana* (Burm. f.) T. Norl. | 92 | S | 7.3 | 35[e] | ..... | ..... | ..... | ..... | 16 |
| *Chrysanthemoides monilifera* (L.) T. Norl. | 92(3) | S | 10.8(3) | 50(3)[e] | ..... | 146(2) | 1.4900(2) | 0(2)(A) | 38(3) |
| *Dimorphotheca chrysanthemifolia* DC. | 80 | S | 2.4 | 48 | 28 | 138 | 1.4894 | 70(B) | 2 |
| *Dimorphotheca cuneata* Less. | 73 | S | 4.6 | 32 | 40 | 137 | 1.4905 | 69(B) | 3 |
| *Dimorphotheca pluvialis* (L.) Much. | 55(6) | S | 2.0(6) | 40(6) | 32(6) | 136(4) | 1.4884(4) | 67(5)(B) | 3(5) |
| *Dimorphotheca sinuata* DC. | 58(13) | S | 1.1(16) | 37(15) | 37(12) | 135(2) | 1.4884(2) | 59(8)(B) | 3(6) |
| *Dimorphotheca zeyheri* Sond. | 80 | S | 2.6 | 36 | 42 | 130 | 1.4805 | 43(B) | 2 |
| *Osteospermum amplectans* (Harv.) T. Norl. | ..... | S + P | 4.5 | 17 | 13 | 144 | 1.4860 | 6(A) | 30 |
| *Osteospermum asperulum* (DC.) T. Norl. | ..... | S + P | ..... | 10[e] | ..... | ..... | ..... | ..... | 29 |
| *Osteospermum calendulaceum* L.f. | 84 | S | 1.5 | 52 | ..... | ..... | 1.4832 | 1(A) | 31 |
| *Osteospermum caulescens* Harv. | ..... | S + P | 15.1 | 14 | 13 | 133 | 1.4851 | 55(B) | 2 |
| *Osteospermum clandestinum* (Less.) T. Norl. | 88(2) | S | 1.8(2) | 42(2) | 38(2) | 153 | 1.4968 | 3(2)(A) | 48(2) |
| *Osteospermum corymbosum* L. | ..... | S + P | ..... | 9[e] | ..... | ..... | ..... | ..... | 23 |
| *Osteospermum dregei* (DC.) T. Norl. | ..... | S + P | 3.6 | 15 | 9 | 135 | 1.4877 | 64(B) | 2 |
| *Osteospermum ecklonis* (DC.) T. Norl. | 64(2) | S | 7.7(2) | 49(2) | 27(2) | ..... | ..... | 67(B) | 2 |
| *Osteospermum fruticosum* (L.) T. Norl. | ..... | S + P | ..... | 14[e] | ..... | ..... | ..... | 61(B) | 2 |
| *Osteospermum hyoseroides* (D.C.) T. Norl. | 82 | S | 3.9 | 50 | 35 | 143 | 1.4871 | 1(A) | 41 |
| *Osteospermum imbricatum* L. | ..... | S + P | ..... | 6(2)[e] | ..... | ..... | ..... | ..... | 19(2) |
| *Osteospermum imbricatum* ssp. *nervatum* (DC.) T. Norl. | ..... | S + P | ..... | 11[e] | ..... | ..... | ..... | ..... | 19 |
| *Osteospermum jucundum* (E. P. Phill.) T. Norl. | ..... | S + P | ..... | 18[e] | ..... | ..... | ..... | 71(B) | 2 |
| *Osteospermum junceum* Berg. | 81 | S | 20.7 | 61 | 26 | 136 | 1.4851 | 0(A) | 32 |
| *Osteospermum microphyllum* DC. | 90 | S | 4.6 | 37 | ..... | ..... | 1.4795 | 1(A) | 21 |
| *Osteospermum muricatum* E. Mey. ex DC. | ..... | S + P | 6.2(5) | 9(5) | 12(4) | 141(2) | 1.4853 | 1(5)(A) | 30(5) |
| *Osteospermum scariosum* DC. | 89(3) | S | 2.7(3) | 41(3) | 37(2) | 141(2) | 1.4857(3) | 0(3)(A) | 34(3) |
| *Osteospermum sinuatum* (DC.) T. Norl. | 85(6) | S | 3.7(6) | 39(6) | 44(6) | 140(4) | 1.4835(4) | 2(6)(A) | 32(6) |
| *Osteospermum spinescens* Thunb. | ..... | S + P | ..... | 6(2) | ..... | ..... | ..... | ..... | 27(2) |
| | 85(3) | S | 3.2(3) | 42(3) | 41(2) | 149 | 1.4891(2) | 2(3)(A) | 38(3) |
| *Osteospermum spinosum* L. | ..... | S + P | ..... | 7(2) | ..... | ..... | ..... | ..... | 44(2) |

[a] Whenever more than one sample of a species was analyzed, the number is shown in parentheses, and the value is the average of those obtained.
[b] S, seed; S + P, seed + pericarp.
[c] Wt per 1,000 units analyzed.
[d] (A) By hydrogen bromide titration; (B) by UV analysis.
[e] Moisture content assumed.

TABLE II

GLC Data on Methyl Esters from Selected Calenduleae Oils

| Species | Methyl ester composition, % | | | | | | |
|---|---|---|---|---|---|---|---|
| | GLC | | | | | | UV[a] |
| | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 conj. | Other | 18:3 conj. |
| *Calendula officinalis* (2)[b] | 5 | 2 | 5.5 | 34 | 53 | 0.5 | 59 |
| *Chrysanthemoides monilifera* (3)[b] | 6 | 7 | 16 | 37 | 33 | 2 | 38 |
| *Crysanthemoides incana* | 10 | 5 | 17 | 56 | 11 | 1 | 16 |
| *Osteospermum amplectans* | 4 | 4 | 10 | 52 | 29 | 1 | 30 |
| *Osteospermum microphyllum* | 9 | 6 | 24 | 39 | 20 | 1 | 21 |
| *Osteospermum spinescens* | 6 | 4 | 8 | 46 | 34 | 1 | 39 |

[a] Determined on oil.
[b] Number of samples included in the average figures shown.

dimorphecolic acid, the isomer having the hydroxyl group at position 13 and the unsaturation at positions 8 and 10. Our survey analyses do not distinguish between isomers of this type, nor do they detect the small amounts of epoxy compounds reported by Morris et al. (12) in *Dimorphotheca.*

The conjugated trienoic acid in *Calendula officinalis* oil was shown by McLean and Clark (9) to be the 8,10,12 isomer and by Chisholm and Hopkins (1) to have the *trans,trans,cis* configuration. Although no definitive evidence has been obtained, it is reasonable again (4) to suggest that the conjugated trienes here reported may be the 8,10,12 isomer which arises in one way or another in the plant by dehydration of dimorphecolic acid. However, should this be the mechanism, it would involve the unexpected transformation of the 12,13 double bond from *trans* to *cis* to make the configuration like that in *Calendula.* Since Hopkins and Chisholm (7) report that at least one genus can produce two different conjugated trienoic acids, the question can be resolved only by further investigation.

In any case, although effects of soil and climate on composition cannot be discounted, present analyses indicate the existence of a wealth of genetic stock from which oilseeds rich in either dimorphecolic acid or conjugated trienoic acid might be developed.

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# Autoxidation of Fatty Materials in Emulsion. II. Factors Affecting the Histidine-Catalyzed Autoxidation of Emulsified Methyl Linoleate[1]

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## Abstract

Several factors which affect autoxidation of methyl linoleate in emulsion have been examined. Data are presented which indicate: 1) In the presence of histidine, the ionic (anionic) emulsifiers examined promote autoxidation of emulsified methyl linoleate, but nonionic emulsifiers do not. 2) The concentration of an emulsifier affects the rate of oxygen absorption. 3) Inorganic salts (0.1 M or less) such as sodium chloride, sodium acetate and sodium sulfate affect oxygen absorption of emulsified methyl linoleate prepared with either inonic or nonionic emulsifiers. In histidine-catalyzed autoxidation there is a suppressing effect in the case of the ionic and a promotional effect in the case of the nonionic. In uncatalyzed autoxidation, these salts have a promotional effect in ionic emulsions and none in nonionic emulsions. 4) Sodium phosphate buffers completely suppress autoxidation due to histidine catalysis, but do not suppress the normal uncatalyzed autoxidation of emulsified methyl linoleate. 5) The pro-oxidative effects of histidine and histidine-metal ion complexes on emulsified unsaturated materials is not limited to polyolefins but also includes mono-olefinic compounds.

## Introduction

IN A PREVIOUS paper (7) the pro-oxidative effects of histidine and metal ions on the autoxidation of linoleate esters in emulsion were reported. Although in that report cognizance was given to other factors which also affect autoxidation in emulsion, data and details were then incomplete.

One such factor then under investigation was whether the type and concentration played any role in the autoxidation process other than aiding in emulsion formation. The question as to whether emulsifiers have a promotional, retarding, or neutral effect upon autoxidation processes has not been studied in detail. However, Marcuse (6) observed that the Tweens and several related nonionic emulsifiers generally retarded pro-oxidative effects and enhanced antioxidative ones in proportion to emulsifier concn.

Another such factor then under investigation was the effect of inorganic salts which are often introduced as components of buffer mixtures. It is general knowledge that inorganic salts are used in many

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