Table II. Enzymatic Hydrolysis of Nucleotide Products from Sodium Naphthalene Reductions

Compd	OD_{267} units of sample	Nucleoside:Nucleotide ratio		
		From snake venom enzyme	From spleen enzyme	Calcd
dTpT	10	1.06	0.9	1
dTpTpT-pT	14	3.1	3.5[a]	3
dTpTpT-pTpT	17	3.9	4.0	4

[a] In this experiment 93% of the substrate was hydrolyzed and 7% remained undegraded. In all other cases the initial nucleotide was hydrolyzed completely and only thymidine and thymidine phosphate were found.

(71% based on an extinction coefficient of 36 400 for this tetramer and on starting triester, 1.37 μmol). Another reaction gave a 69% yield of dTpTpTpT.

dTpTpTpTpT. Chain extension of compound **7** (from the second synthetic sequence; 70 mg, 0.038 mmol) was accomplished by reaction with **3** prepared from 5′-*O*-phenoxyacetylthymidine (54 mg, 0.143 mmol) by the same procedure used for constructing **7.** The resulting products were analyzed (after extraction of the 3′–3′ dinucleotide isomer derivative with four portions of 10% ethanol in water and before purification on silica gel) by gel filtration on a Bio-bead column. The elution pattern indicated good conversion to the protected pentamer (Figure 2). Purification of the main portion obtained from the extractions by chromatography on silica gel plates using ether–chloroform–ethanol (1:1:2 v/v/v, four developments) afforded 1235 OD_{267} units (69% based on an extinction coefficient of 46 900) of purified pentamer: λ_{max} 267 nm and λ_{min} 243 nm; R_f (THF) 0.05; R_f($CHCl_3$–C_2H_5OH–$C_2H_5OC_2H_5$ 1:1:1) 0.06. Deblocking by use of sodium naphthalene in HMPA as described above, using 101 OD_{267} units of the protected pentamer, yielded 57.5 OD_{267} units (59%) of dTpTpTpTpT; R_m^{dpT} 0.81; R_f (solvent A) 0.012, R_f (solvent F) 0.30. The elution pattern for fractionation of the reaction products is given in Figure 3.

Enzymatic Degradation. The nucleotide products were hydrolyzed by use of snake venom phosphodiesterase and by spleen phosphodiesterase by conventional procedures.[23] The products were characterized by paper electrophoresis and chromatography (solvent A). For quantitative analysis, they were eluted from the paper (solvent A) and determined by the absorbance at 267 nm. The results are given in Table II.

References and Notes

(1) This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health (GM 10265).
(2) For a recent comprehensive review, see H. Kössel and H. Seliger in *Prog. Chem. Org. Nat. Prod.*, **32**, 297 (1975).
(3) See R. C. Pless and R. L. Letsinger, *Nucleic Acids Res.*, **2**, 773 (1975), and H. Koster, A. Pollak, and F. Cramer, *Justus Liebigs Ann. Chem.*, 959 (1974), for recent examples of syntheses on polymer supports.
(4) R. L. Letsinger and G. H. Heavner, *Tetrahedron Lett.*, **2**, 147 (1975).
(5) C. B. Reese and R. Saffhill, *Chem. Commun.*, 767 (1968).
(6) F. Eckstein and I. Rizk, *Angew. Chem.*, **79**, 684 (1967).
(7) C. B. Reese, *Colloq. Int. C. N. R. S.*, **No. 182**, 319 (1970).
(8) R. C. Pless, Ph.D. Dissertation, Northwestern University, Evanston, Ill., 1973.
(9) Observation of G. A. Heavner.
(10) Observation of J. L. Finnan.
(11) R. L. Letsinger, J. L. Finnan, G. A. Heavner, and W. B. Lunsford, *J. Am. Chem. Soc.*, **97**, 3278 (1975).
(12) For use of phenoxyacetyl as an O-protecting group for ribonucleosides, see C. B. Reese and J. C. M. Stewart, *Tetrahedron Lett.*, 4273 (1968).
(13) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Am. Chem. Soc.*, **88**, 829 (1966).
(14) J. H. van Boom, P. M. J. Burgers, G. R. Owen, C. B. Reese, and R. Saffhill, *Chem. Commun.*, 869 (1971).
(15) This result indicates, as anticipated, that the *o*-chlorophenyl phosphorodichloridite is much more reactive than monochloridite **3.**
(16) T. Nielson, private communication.
(17) G. L. Greene and R. L. Letsinger, *Tetrahedron Lett.*, 2081 (1975).
(18) R. L. Letsinger and W. S. Mungall, *J. Org. Chem.*, **35**, 3800 (1970).
(19) Caution: Since completion of this work, HMPA has been reported to cause cancer in experimental animals. Use of other solvents for this step is under investigation.
(20) H. Tolksmith, *J. Org. Chem.*, **23**, 1682 (1958).
(21) W. Gerrard, W. J. Green, and R. J. Phillips, *J. Chem. Soc.*, 1148 (1954).
(22) K. K. Ogilvie and R. J. Letsinger, *J. Org. Chem.*, **32**, 2365 (1967).
(23) W. E. Razzell and H. G. Khorana, *J. Biol. Chem.*, **236**, 1144 (1961); R. K. Ralph, W. J. Connors, H. Schaller, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 1983 (1963).
(24) Compound **4a** was prepared by J. Finnan by a procedure similar to that used in making **4b** and the products were separated prior to alkaline hydrolysis.
(25) R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, **91**, 3350 (1969).

Synthesis of the Witchweed Seed Germination Stimulant (+)-Strigol[1-3]

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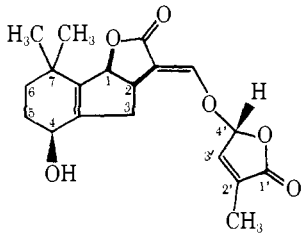
Abstract: A synthesis of (±)-strigol (**24**) and (±)-4′-epistrigol (**25**) has been carried out by formylation of (±)-1α,4α-dihydroxy-7,7-dimethyl-4,5,6,7-tetrahydroindan-2α-acetic acid γ-lactone (**21**) followed by alkylation of the resulting hydroxymethylene lactone (**2**) with the bromobutenolide **4.** Similarly prepared were (±)-4-epistrigol (**25**) and (±)-4,4′-diepistrigol (**26**). Resolution of (±)-**21** was accomplished by its conversion into the diastereomeric 3β-acetoxyandrost-5-ene-17β-carboxylate derivatives. Using the same reaction sequence as for the racemic series, (+)-**21** was converted into (+)-strigol and (+)-4′-epistrigol while (−)-**21** yielded (−)-strigol and (−)-4′-epistrigol. Routes to **21** from both α-cyclocitral (**11a**) and β-cyclocitral (**11b**) via the common intermediate methyl 3-oxo-2,6,6-trimethylcyclohex-1-ene-1-carboxylate (**9b**) are described.

Witchweed (*Striga lutea* Lour.) is an angiospermous root parasite that attacks corn, sorghum, sugarcane, rice, and more than 60 other important crop plants and weeds of the grass family.[4] *Striga* species occur commonly in the Eastern Hemisphere and inflict serious crop damage in many parts of the world.[4,5] The discovery of witchweed in the United States in 1956,[6] the first report of this pest in the Western Hemisphere, has been a matter of concern.

The germination of the seeds of witchweed, which may lie dormant for 15–20 years, is induced by a stimulant released by the roots of the host plant.[5] This stimulant is also released by certain other plant species which are not parasitized by witchweed.[7-9] Cook et al.[10] isolated this germination stimulant, strigol, from the root exudates of cotton (*Gossypium hirsutum* L.) and determined its structure and relative configuration as depicted in **1**[11] largely by spectroscopic and x-ray crystallo-



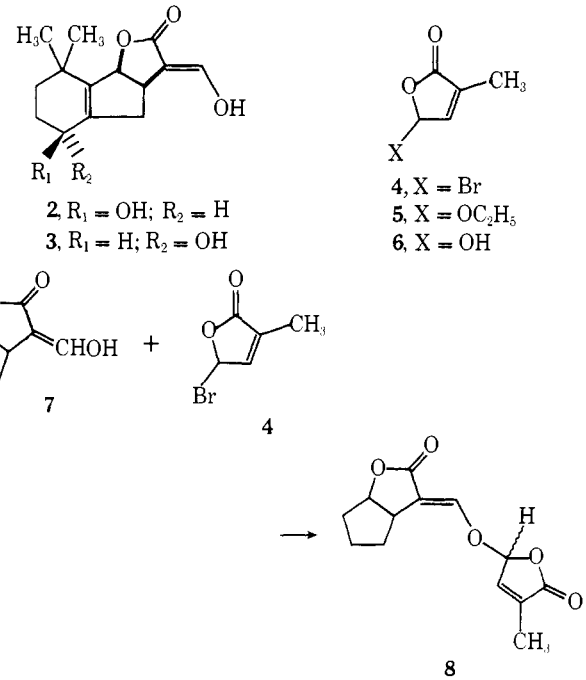
1

graphic data.[12] Strigol is an extremely active compound, concentrations of 10^{-11} M being capable of stimulating seed germination, and it has been suggested that strigol may be representative of a new class of plant hormones.[12]

Biological studies with strigol have been impeded by its unavailability, since the natural material can be obtained in only minute quantities after tedious isolation and purification.[10] We therefore endeavored to devise a practical, chemical synthesis of this molecule to make it available in quantities sufficient for investigative purposes. Preliminary reports of the synthesis of racemic strigol were communicated from our laboratory[1] and elsewhere.[13] We now report the experimental details successfully employed for the preparation of gram quantities[14] of *dl*-strigol and its isomers as well as the method of resolution employed for the synthesis of strigol in its optically active enantiomeric forms.

In approaching the synthetic problems presented by strigol, we viewed the molecule as a condensation product of hydroxymethylene lactone **2** and an alkylating agent such as bromobutenolide **4.** As the stereochemistry of O-alkylation of β-dicarbonyl compounds can be strongly influenced by solvent properties,[15] it seemed likely that the stereochemistry about the enol ether bond could be influenced to give a favorable ratio of the *E* and *Z* isomers. To test this crucial final step in the synthetic sequence, we examined the alkylation of the model hydroxymethylene lactone **7**[16] with the bromobutenolide **4,** which was prepared by a three-step process: photochemical oxygenation of 3-methyl-2-furoic acid[17] in ethanol[18] with subsequent stannous chloride reduction of peroxides gave lactone acetal **5**;[19] hydrolysis of **5** in boiling water yielded lactol **6**;[19] and treatment of **6** with carbon tetrabromide and triphenylphosphine[20] gave **4** in 47–50% overall yield.

The alkylation of the model lactone **7** with **4** under the initially tried conditions (K_2CO_3 in hexamethylphosphoric triamide at ambient temperature) proved to be highly selective for the desired *E* isomer, the only product detected being the diastereomeric mixture **8** (90% yield). The stereochemical assignment was based on the NMR signal for the alkoxymethylene vinyl proton of **8,** which appeared as a doublet (J = 2.4 Hz) at δ 7.37. This value is in good agreement with the analogous highly deshielded proton in strigol at δ 7.42 (d, J = 2.4 Hz).[12] The assignment of *E* stereochemistry to the alkylation product **8** has been supported by subsequent reports.[13,21] No effort was made to separate these diastereomers.



2, R_1 = OH; R_2 = H
3, R_1 = H; R_2 = OH

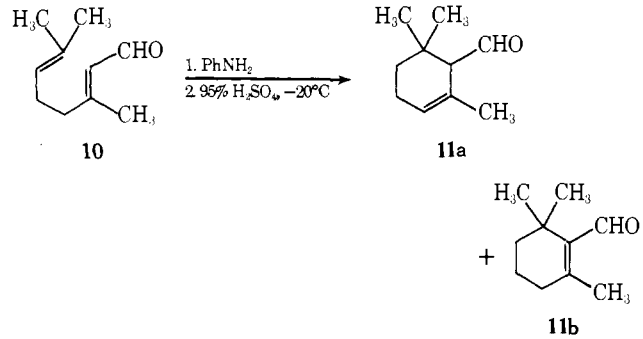
4, X = Br
5, X = OC_2H_5
6, X = OH

7

4

8

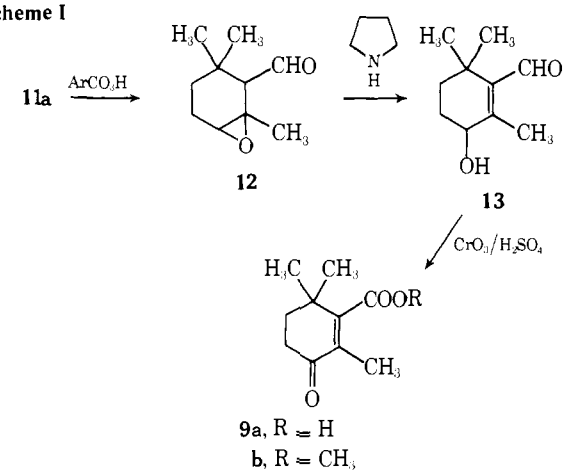
With the assurance afforded by this favorable finding, the synthetic problem was essentially reduced to the preparation of hydroxymethylene lactone **2.** We considered that this synthetic fragment could be derived from methyl 2,6,6-trimethylcyclohex-1-en-3-one-1-carboxylate (**9b**) with citral (**10**) as the ultimate starting material. Proceeding by known methods,[22] citral was converted to its anil and was cyclized with sulfuric acid at −20 °C to afford a mixture of α-cyclocitral (**11a**) and β-cyclocitral (**11b**). As had been previously noted,[23] it was possible to alter the product ratio of these two isomers. Conducting the reaction under an atmosphere of air produced a 60% yield of cyclocitrals with the ratio of **11a**/**11b** being 1:3 (reported[23] 48%; 2:1), whereas conducting the reaction under nitrogen altered the ratio of **11a**/**11b** to 10:1 (reported[23] 15:1). Since α-cyclocitral is readily converted to β-cyclocitral by base treatment, either **11a** or **11b** (94%, reported[23] 72%) could be produced in good yield from citral, and both were used in separate routes to **9b.**



10 → 11a + 11b

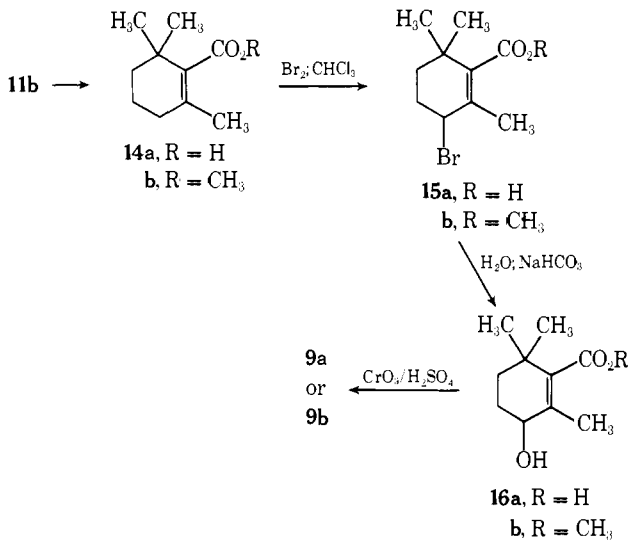
In the first route (Scheme I) α-cyclocitral (**11a**) was treated with *m*-chloroperoxybenzoic acid to give the epoxide **12** (90% yield), which was then reacted with pyrrolidine in ether to give hydroxy aldehyde **13** in 76% yield. Oxidation of **13** with Jones reagent[24] afforded **9a** in 45–55% yield accompanied by 20–30% of material in which the highly hindered aldehyde function remained unoxidized. While the hindered carboxylic acid function *in* **9a** *remained unaffected* by both methanol–sulfuric

Scheme I

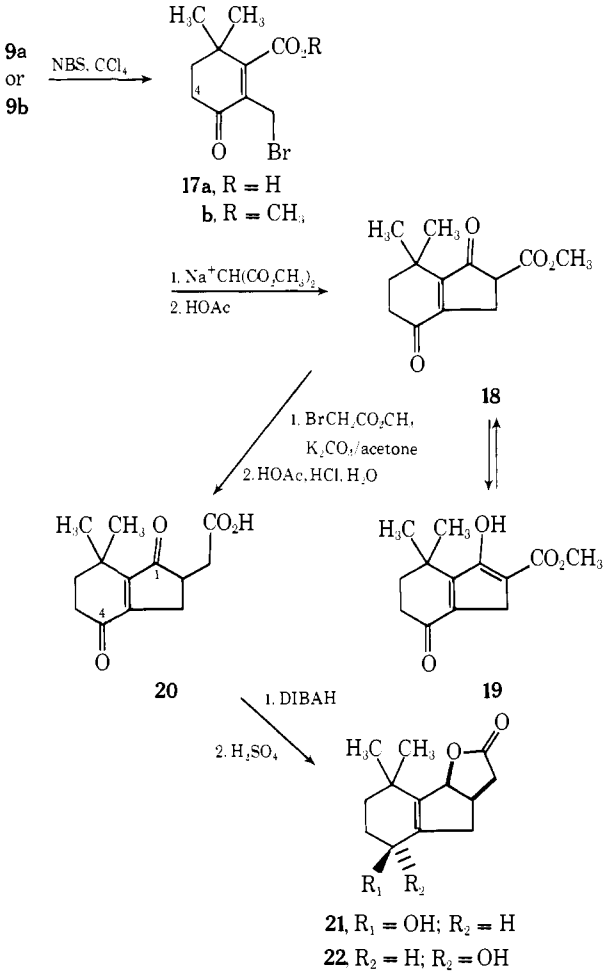


9a, R = H
b, R = CH_3

Scheme II



Scheme III



acid and methanol–boron trifluoride etherate,[25] esterification was achieved with methyl iodide and potassium carbonate giving **9b** in quantitative yield.

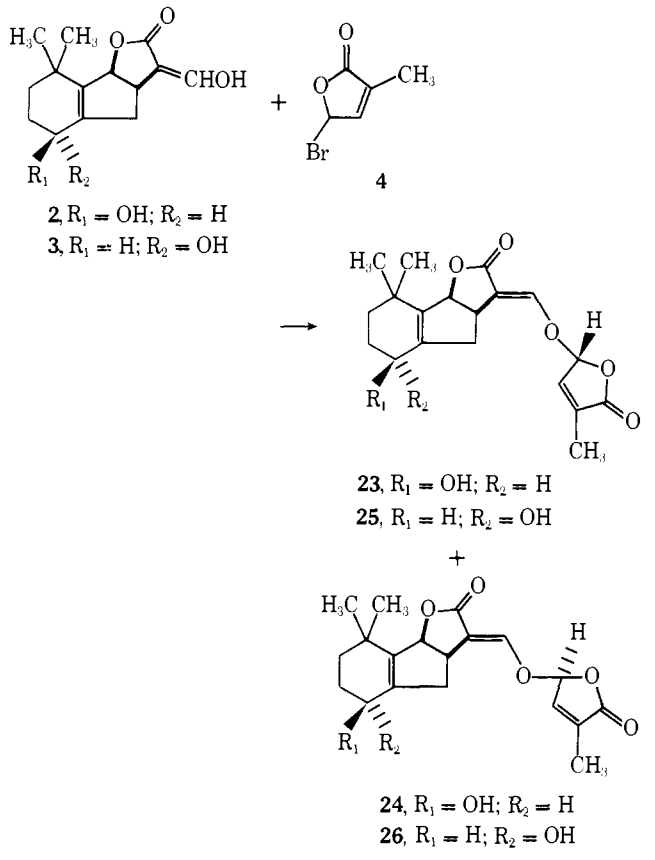
The second route to **9b** (Scheme II) proceeded from β-cyclocitral (**11b**) and was originally an attempt to repeat a published procedure.[26] These efforts were frustrated at every step by yields substantially lower than those reported. However, by various modifications, an acceptable route to **9b** was developed. In the first step, vigorous agitation of **11b** under an oxygen atmosphere gave the acid **14a** in 45–50% yield with no recovered starting material (reported[26] 62.5% yield with 37.5% recovered **11b**). To improve this step, the reaction was conducted in the presence of 5% platinum on carbon at reduced temperatures, giving **14a** in 70% yield. Bromination of **14a** gave **15a** in 45–50% yield (reported[26] 65–70%), and hydrolysis of **15a** in boiling water gave **16a** in 70% yield (reported[26] 91%). Jones oxidation[24] then gave **9a** in 95% yield. The disappointing yield of the bromination step was markedly improved by first converting **14a** into its methyl ester **14b** with methyl iodide and potassium carbonate. Reaction with bromine in chloroform then afforded in nearly quantitative yield the bromide **15b** which was hydrolyzed to **16b** in 70% yield. At this stage it was mandatory to purify **16b** chromatographically to remove small amounts of impurities which seriously interfered with a subsequent step (vide infra). Jones oxidation[24] of **10b** gave **9b** quantitatively.

The first step in the conversion of **9b** to hydroxymethylene lactone **2** (Scheme III) consisted of functionalization of the C-2 methyl group to enable construction of the five-membered ring. Thus, the keto ester **9b** was treated with *N*-bromosuccinimide in carbon tetrachloride to give the bromide **17b** in nearly quantitative yield with no evidence of competitive bromination at C-4 adjacent to the carbonyl group. When **9b** from the second method of preparation (Scheme II) was used in this step without purification at the stage of **16b,** the reaction totally failed. Purified material behaved normally, however. It was presumed that the unidentified impurities functioned as efficient radical traps and blocked the reaction path. The acid **9a** could also be efficiently brominated to **17a,** although this material was less suitable for subsequent transformations due to a tendency to internally alkylate the carboxylic acid to form a lactone. In the case of **9a,** chromatographic purification of precursors was unnecessary since both **16a** and **9a** were isolated as crystalline solids.

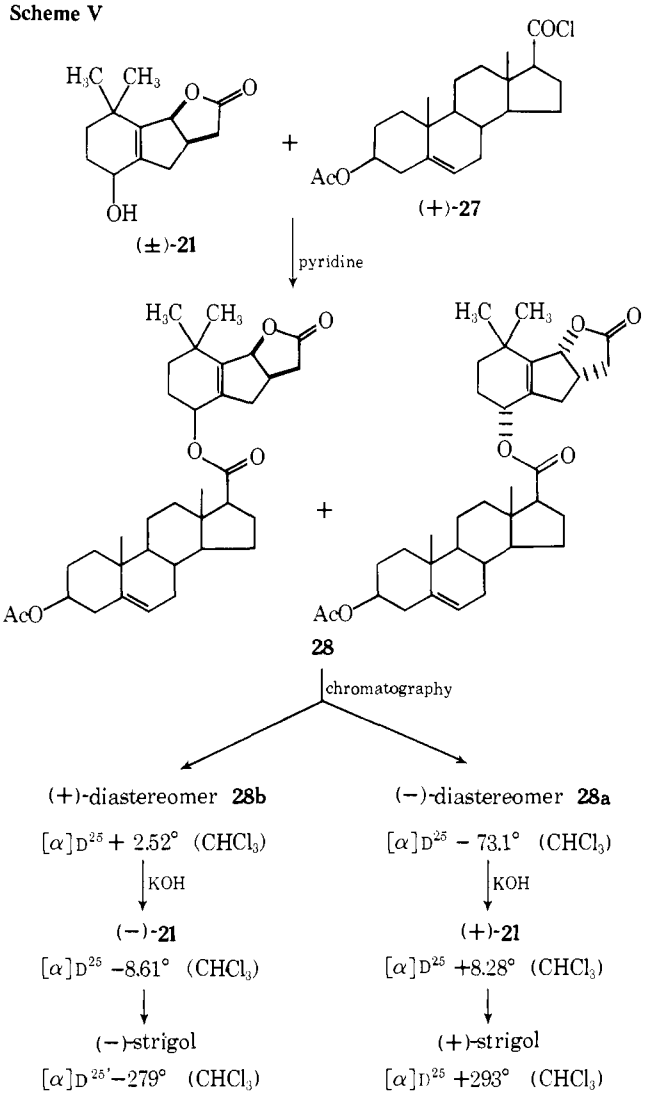
To form the five-membered ring, **17b** was reacted with excess dimethyl sodiomalonate in methanol resulting in alkylation and cyclization to give a mixture of the β-keto ester **18** and its enolic tautomer **19** in 80–86% yield. The ratio of these tautomers in the solid product varied from run to run, and while we were not able to obtain the keto form **18** free of its tautomer, we were able to recrystallize selected samples to obtain the enol form **19** essentially free of the keto form.

This mixture of **18** and **19** was alkylated with methyl bromoacetate in the presence of potassium carbonate, and the alkylation product was heated with hydrochloric acid in acetic acid, resulting in hydrolysis and decarboxylation to give the diketo acid **20** in 72% yield. A variety of reducing agents ($LiAlH_4$, $NaBH_4$, borane–tetrahydrofuran, catechol–borane, sodium bis(2-methoxyethoxy)aluminum hydride, lithium tri-*tert*-butoxyaluminum hydride, potassium tri-*sec*-butylborohydride, H_2–PtO_2, and zinc–chlorotrimethylsilane) were tried in an attempt to reduce the carbonyl functions at C-1 and C-4 of **20** or its corresponding methyl ester. The general result of these efforts was either a complex mixture of products or simply the reduction of the C-4 carbonyl with the highly hindered carbonyl at C-1 remaining intact. However, a very modest yield of lactones **21** and **22** was obtained by reduction of **20** with borane–tetrahydrofuran. The exception to this general failure was diisobutylaluminum hydride (DIBAH), which cleanly reduced **20** in methylene chloride. After acidification and column chromatography on silicic acid, a mixture of hydroxy lactones **21** and **22** (ca. 1:1) was obtained in 63% yield. This purified mixture could be crystallized to give **21** in 26% yield; the mother liquor was then chromatographed on alumina to give **22** as an oil and additional quantities of **21.** With reductions on a scale of 10 g or more, **21** could be isolated in 22% yield by direct crystallization from the crude product mixture without resorting to a prior cleanup chromatographic purification. For best results, it was important to use 3.5 equiv

Scheme IV



Scheme V



of DIBAH and to maintain the temperature initially at −70 °C or less. While the possibility exists for recycling **22** to give additional amounts of **21,** this scheme was not explored.

For the final stages of the synthesis (Scheme IV), **21** was condensed with methyl formate in the presence of sodium hydride to give **2** (78% yield), which was then alkylated with bromobutenolide **4,** under the same conditions used for the model compound **7,** to give a mixture of the two diastereomers **23** and **24.** This mixture was separated chromatographically giving **23** in 27% yield and **24** in 18% yield. These diastereomers were essentially identical by NMR, ir, uv, and mass spectra and differed only in their melting points and chromatographic behavior. On thin layer chromatography (silica gel, chloroform–acetone, 4:1) the slower moving isomer had an R_f value of 0.2 and migrated at the same rate as an authentic sample of natural strigol,[27] while the faster moving isomer had an R_f value of 0.32. The melting point of the slower moving isomer (mp 203–205 °C dec) was also in agreement with its assignment as (±)-strigol (**23**) since it compared favorably with that of natural (+)-strigol (reported[12] mp 200–202 °C dec) while the melting point of the faster moving isomer (mp 178–180 °C) differed considerably and was thus assigned the structure of (±)-4′-epistrigol (**24**). Furthermore, the relative biological potencies of the two isomers were in agreement for their assigned structures: concentrations required for 50% seed germination (*Striga lutea* Lour.) were 10^{-12} M for (±)-4′-epistrigol (**24**) and 10^{-16} M for (±)-strigol (**23**).[28]

In a similar fashion, **22** was condensed with methyl formate to afford **3** (75% yield), which was likewise alkylated with **4** to give a mixture of (±)-4-epistrigol (**25**) and (±)-4,4′-diepistrigol (**26**). These diastereomers were separated chromatographically, and both showed ir, uv, and mass spectra identical with those for **23** and **24.** However, the NMR spectra for **25** and **26** (identical with each other) differed somewhat from those for **23** and **24,** particularly in the position and multiplicity of the signal of the C-3 protons.

After completing the synthesis of (±)-strigol, we turned to the problem of preparing strigol in its optically active forms and to establish which of the two antipodes, (+) or (−), corresponds to natural strigol. Another goal of this phase of the work was to determine the absolute stereochemistry of the natural form of strigol. Resolution was accomplished at the stage of hydroxylactone **21** (Scheme V) by forming diastereomeric esters on reaction of racemic **21** with (+)-3β-acetoxyandrost-5-ene-17β-carboxylic acid chloride (**27**) in pyridine. The diastereomers **28** were separated by chromatography, and the esters were hydrolyzed with potassium hydroxide in methanol to give, after chromatography on alumina, (+)-hydroxy lactone **21** (αD +8.28°) and (−)-hydroxy lactone **21** (αD −8.61°).

Following the synthetic sequence used for the elaboration of racemic strigol, (−)-**21** was formylated and condensed with bromobutenolide **1** yielding the expected two diastereomeric strigols, which were chromatographically separated. One of these isomers, (−)-strigol (mp 193–194 °C), possessed the chromatographic mobility and spectral properties of natural strigol, but its circular dichroism (CD) curve was clearly that of the optical antipode. The second isomer, mp 165–166 °C, was identified as (−)-4′-epistrigol, as it possessed the same chromatographic behavior as the racemic modification. Similarly, (+)-**21** was formylated with methyl formate and alkylated with **4** to yield another diastereomeric pair, again separated chromatographically. One of these isomers was characterized as (+)-4′-epistrigol. The second isomer, mp

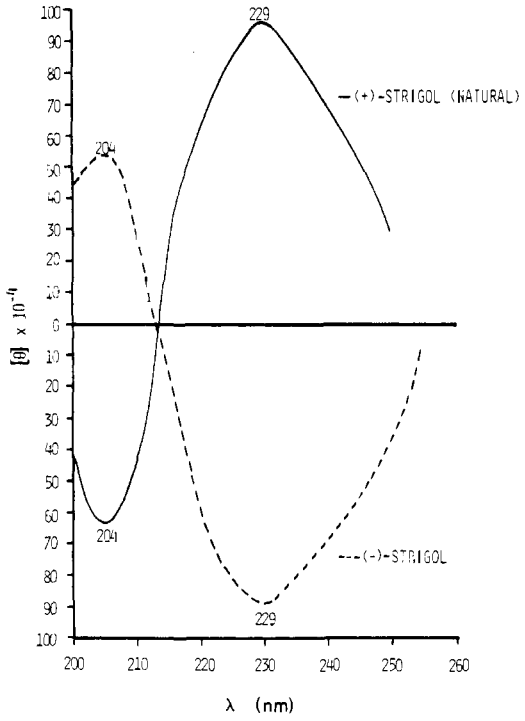
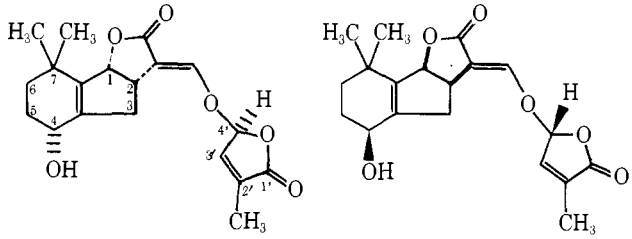


Figure 1. The CD curves of (+)-strigol (2.31×10^{-4} M, naral configuration) and (−)-strigol (1.84×10^{-4} M) in methanol.

200–202 °C, was characterized as (+)-strigol since its uv, ir, NMR, CD,[27] and mass spectra were identical with those of natural strigol. The melting point and chromatographic behavior were also supportive. The CD curves of synthetic (+)-strigol (natural configuration) and (−)-strigol are shown in Figure 1.

The question which remained was whether the absolute configuration of (+)-strigol was 4*R*,4′*S* or 4*S*,4′*R* as depicted below. We attempted to resolve this question by the application of the methods of Nakanishi[29] and Horeau[30] to hydroxy lactones (−)-**21** and (+)-**21**. Figure 2 represents the CD curve of enantiomer (−)-**21** induced by copper hexafluoroacetylacetonate ($Cu(hfac)_2$).[29] Based on the signs of the CD extrema in the 305–355-nm region, one would surmise that (+)-strigol bears the 4*R*,4′*S* configuration. However, analysis of these



(4*R*,4′*S*) (4*S*,4′*R*)

hydroxy lactones using Horeau's method[30] led to the opposite conclusion. When (+)-hydroxy lactone (**21**) was acylated with an excess of α-phenylbutyric anhydride, the rotation of the recovered α-phenylbutyric acid was (−), indicating *S* configuration for (+)-**21**. Conversely, (+)-α-phenylbutyric acid was recovered after acylation of (−)-**21**. Thus, it appears that the problem of absolute configuration of (+)-strigol may best be settled by x-ray crystallography. Preparation of an ester derivative of (−)-**21** containing a heavy atom is currently in progress.

Experimental Section[31]

4-Bromo-4-hydroxy-2-methyl-2-butenoic Acid Lactone (4). A solution of 3.09 g (27 mmol) of 4,4-dihydroxy-2-methyl-2-butenoic acid

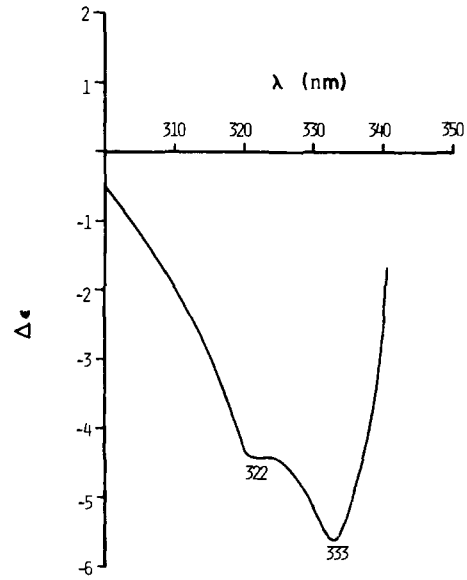


Figure 2. The induced CD resulting from a mixture of 1.04×10^{-4} M $Cu(hfac)_2$ and 9.0×10^{-4} M (−)-hydroxy lactone **21** in CCl_4.

lactone[18,19] (**6**) and 11.2 g (34 mmol) of carbon tetrabromide in 35 ml of dry dichloromethane, stirred under nitrogen with cooling in an ice–water bath, was treated with a solution of 7.8 g (30 mmol) of triphenylphosphine in 20 ml of dichloromethane by dropwise addition over a period of 40 min. After stirring for an additional 5 h at 0°, the liquid was decanted, and the solid triphenylphoshpine oxide was rinsed twice with ether. The decanted liquid and ether rinses were combined and chilled in an ice–water bath to crystallize out additional amounts of triphenylphosphine oxide. After again decanting and rinsing with ether, the solvents were evaporated, and the residue was distilled in vacuo to give bromobutenolide **4** (3.25 g, 68%): bp 54 °C (0.4 mm); ir (film) 1778 (C=O), 1650 cm^{-1} (C=C); uv_{max} (95% C_2H_5OH) 212 nm (ε 12 600); NMR ($CDCl_3$) δ 1.98 (3, t, *J* = 1.6 Hz), 6.83 (1, pentet, *J* = 1.6 Hz), 7.20 (1, pentet, *J* = 1.6 Hz). The compound was too unstable for elemental analysis.

Alkylation of Hydroxymethylene Lactone 7 with Bromobutenolide 4. To a mixture of 308 mg (2.0 mmol) of 1′-(hydroxymethylene)-2α-hydroxycyclopentane-1α-acetic acid γ-lactone (**7**)[16] and 290 mg (2.1 mmol) of anhydrous potassium carbonate in 2 ml of hexamethylphosphoric triamide, stirred under a nitrogen atmosphere at ambient temperature, was added 410 mg (2.3 mmol) of bromobutenolide **4**. The reaction mixture was stirred for 30 h at ambient temperature and was then diluted with water and extracted three times with ethyl acetate. The combined extracts were washed three times with water and once with saturated NaCl solution. The dried (Na_2SO_4) extracts were concentrated under reduced pressure to give 460 mg (92%) of oily product **8**, which was nearly pure by NMR analysis. Column chromatography (silica gel, ethyl acetate–benzene gradient) afforded pure material: ir ($CHCl_3$) 1782, 1737, 1675 cm^{-1}; NMR ($CDCl_3$) δ 1.72 (6, complex), 1.98 (3, t, *J* = 1.6 Hz), 3.47 (1, m), 4.95 (1, m), 6.17 (1, pentet, *J* = 1.6 Hz), 7.37 (1, d, *J* = 2.4 Hz).

2,6,6-Trimethylcyclohex-1-ene-1-carboxaldehyde (11b). To a solution of 100 g of KOH in 1.5 l. of methanol, stirred under nitrogen at 0–5 °C, was added all at once 333 g (2.19 mol) of a mixture of α- and β-cyclocitrals (**11a** and **11b**, ratio ca. 10:1). After stirring for 12 min, the mixture was poured into 2 l. of 10% NaCl solution. The mixture was extracted with ether, and the combined ether extracts were washed with water and saturated NaCl solution. The ether layer was dried (Na_2SO_4) and concentrated, and the residue was distilled in vacuo, collecting the fraction boiling at 90–94 °C (12 mm) (313 g, 94% yield; lit.[22] 91–93 °C (12 mm)). NMR analysis indicated the product to be ca. 4% **11a** and 96% **11b**.

2,3-Epoxy-2,6,6-trimethylcyclohexane-1-carboxaldehyde (12). A solution of 307 g (1.77 mol) of *m*-chloroperbenzoic acid, technical, 85% in 2 l. of methylene chloride was added over 30 min to a solution of 204.5 g (1.21 mol) of 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde (**11a**) in 0.5 l. of methylene chloride maintaining a temperature of 20–30 °C. Stirring was continued for an additional 2 h, whereupon

excess peracid was destroyed by addition of 500 ml of 10% aqueous Na_2SO_3 solution. The mixture was made alkaline by the addition of aqueous Na_2CO_3 solution, and the methylene chloride layer was separated and washed with water and saturated NaCl solution. After drying (Na_2SO_4) and concentrating, 217 g (96%) of crude epoxide **12** was obtained and used in the subsequent transformation without further purification.

In a separate experiment, the product was distilled to afford pure epoxide **12:** bp 74–77 °C (1.0 mm); calculated mass for $C_{10}H_{16}O_2$ 168.11503, found *m/e* 168.11497; ir (film) 2715 (O=CH), 1720 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.93 (6, s), 1.15–1.7 (2, m), 1.35 (3, s), 2.05 (3, m), 3.12 (1, t, J = 2 Hz), 9.75 (1, d, J = 5 Hz).

3-Hydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxaldehyde (13). Pyrrolidine (21 ml) was added to a solution of 217 g (1.29 mol) of crude epoxide **12** in 1 l. of ether, and the mixture was stirred under nitrogen at ambient temperature for 3 h. After washing with water and saturated NaCl solution, the ether layer was dried and concentrated. The oily residue was distilled under vacuum giving a forerun and the main fraction of hydroxy aldehyde **13** (157 g, 73% based on **11a**): bp 122–128 °C (1.0 mm) (lit.[32] bp 90–100 °C (bath) (0.001 mm)); calculated mass for $C_{10}H_{16}O_2$ 168.11503, found *m/e* 168.11497; ir (film) 3420 (OH), 1675 (C=O), 1605 cm^{-1} (C=C); NMR (CCl_4) δ 1.15 (3, s), 1.20 (3, s), 1.1–2.1 (4, complex), 2.20 (3, s), 4.08 (1, broadened t, J = 6 Hz), 4.72 (1, s, OH), 10.17 (1, s); uv_{max} (CH_3OH) 245 nm (ϵ 10 100).

3-Oxo-2,6,6-trimethylcyclohex-1-ene-1-carboxylic Acid (9a). Hydroxy aldehyde **13** (157 g, 0.935 mol) was dissolved in 1.5 l. of acetone, and while maintaining a temperature of 0–5 °C, 250 ml of Jones reagent[24] was added over ca. 9 h. After stirring in the cold for an additional 1 h, excess oxidant was destroyed by the addition of 2-propanol. Sufficient water was added with stirring to dissolve the chromium salts, and most of the acetone was removed by distillation under reduced pressure. Water (1 l.) was added, and the mixture was extracted with four 250-ml portions of ether. The combined extracts were washed with water and extracted with 10% aqueous $NaHCO_3$ solution. The basic extract was acidified with 6 N hydrochloric acid and extracted with ether. The ether extract was washed with water and saturated NaCl solution and dried. Concentration under reduced pressure gave 108 g (63.5%) of crude, solid keto acid **9a.** Recrystallization from benzene afforded 85.6 g (50%) of pure **9a:** mp187–189 °C (lit.[26] mp 192 °C).

2,6,6-Trimethylcyclohex-1-ene-1-carboxylic Acid (14a). To a mixture of 500 mg of 5% platinum on carbon and 750 ml of heptane, stirred under oxygen in an ice–water cooling bath, was added dropwise over 2 h 152 g (1.0 mol) of 2,6,6-trimethylcyclohex-1-ene-1-carboxaldehyde (**11b**), while maintaining a temperature of 0–5 °C. Stirring in the cold was continued until O_2 uptake ceased (17 h): total O_2 uptake was ca. 16.3 l. The mixture was filtered through Celite, diluted with ether, and extracted with 5% aqueous NaOH solution. The aqueous extracts were washed with ether, acidified with concentrated HCl, and extracted with ether. The acidic ether extracts were washed with water and saturated NaCl solution. The mixture was dried and concentrated, and the white, solid residue was dried under vacuum to constant weight to give 116.5 g (69.5% yield) of **14a** (mp 90–92 °C) of satisfactory purity for further transformations. Recrystallization from hexane brought the melting point to 102–104 °C (lit.[33] 104–106 °C).

Methyl 2,6,6-Trimethylcyclohex-1-ene-1-carboxylate (14b). A mixture of 1156 g (6.87 moles) of 2,6,6-trimethylcyclohex-1-ene-1-carboxylic acid, 960 g of anhydrous potassium carbonate, 1075 g of methyl iodide, and 4 l. of acetone was stirred at room temperature for 48 h. The mixture was then concentrated under reduced pressure. Water was added to the residue, and the mixture was extracted with ether. The combined ether extracts were washed with water and saturated NaCl solution and dried. Evaporation of the solvent gave 1240 g of methyl ester **14b.** Distillation afforded pure material (1050 g, 83.9% yield): bp 46–48 °C (0.05 mm); ir (film) 1730, 1660 cm^{-1}; NMR (CCl_4) δ 1.07 (6, s), 1.79 (3, s), 1.28–2.16 (6, complex), 3.72 (3, s).

Anal. Calcd for $C_{11}H_{18}O_2$: C, 72.49; H, 9.96. Found: C, 71.90; H, 9.80.

Methyl 3-Bromo-2,6,6-trimethylcyclohex-1-ene-1-carboxylate (15b). A solution of 91.0 g (0.5 mol) of ester **14b** and 500 ml of chloroform was stirred under a nitrogen atmosphere and illuminated with a 100-W tungsten lamp. While maintaining a temperature below 10° with an ice–water bath, a solution of 31 ml (92 g, 0.60 mol) of bromine and 100 ml of chloroform was added dropwise over a period of 2 hr. Stirring was continued at 10° or lower with illumination for another 45 min, and the reaction mixture was then concentrated under reduced pressure to give 133 g (ca. 100%) of crude bromo ester **15b,** which was used in subsequent transformations without purification.

In a separate run, distillation of the crude product afforded the analytical sample: bp 98–100 °C (1.0 mm); calculated mass for $C_{11}H_{17}BrO_2$ 260.04124, found *m/e* 260.04126; ir (film) 1730 cm^{-1}; NMR ($CDCl_3$) δ 1.08 (3, s), 1.16 (3, s), 1.79 (3, s), 1.3–2.4 (4, complex), 3.77 (3, s), 4.63 (1, m).

Methyl 3-Hydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxylate (16b). Crude bromo ester **15b** (358 g, prepared from 250 g (1.37 mol) of ester **14b**) was added to 2.5 l. of boiling water under a nitrogen atmosphere. A solution of 124 g of sodium bicarbonate in 500 ml of water was then added dropwise over 1.5 h to maintain a slightly alkaline pH in the reaction mixture. The mixture was then cooled in an ice–water bath and extracted with ether. The combined ether extracts were washed with water and saturated NaCl solution, dried, and concentrated in vacuo to give 280 g of crude **16b** containing small amounts of bromo ester **15b** and unidentified, nonpolar impurities. The crude product was placed on a 6 × 70 cm column of silica gel, and the column was washed with benzene until the bromo ester and the nonpolar impurities were absent from the washings (as determined by TLC in chloroform–acetone, 9:1). The product **16b** was then eluted with benzene–ethyl acetate (1:1), and the product fractions were pooled and concentrated in vacuo to give 165 g (61%) of hydroxy ester **16b.** Earlier fractions containing both product and impurities were combined and rechromatographed to give an additional 55 g (20%) of **16b** for a total yield of 81% of pure hydroxy ester **16b:** bp 85–89 °C (0.03 mm) (lit.[26] 100 °C (bath) (0.01 mm)); calculated mass for $C_{11}H_{18}O_3$ 198.12559, found *m/e* 198.12556; ir (film) 3400 (OH), 1726 (C=O), 1710 cm^{-1} (sh); NMR ($CDCl_3$) δ 1.10 (6, s), 1.76 (3, s), 1.3–2.1 (4, complex), 2.42 (1, s, -OH), 3.76 (3, s), 3.98 (1, m).

Methyl 3-Oxo-2,6,6-trimethylcyclohex-1-ene-1-carboxylate (9b).

a) By Esterification of Keto Acid 9a. A mixture of 114 g (0.626 mol) of 3-oxo-2,6,6-trimethylcyclohex-1-ene-1-carboxylic acid, 114 g of anhydrous potassium carbonate, 120 ml of methyl iodide, and 1 l. of acetone was stirred at room temperature for 24 h. The mixture was then concentrated under reduced pressure. Water was added to the residue, and the mixture was extracted with ether. The combined ether extracts were washed with water and saturated NaCl solution and dried (Na_2SO_4). Evaporation of the solvent gave 122 g of methyl ester **9b** of satisfactory purity for further transformation. Distillation gave pure material (121 g, 98.5% yield): bp 72.5–74.5 °C (0.02 mm); ir (film) 1727, 1678, 1620, 1430, 1312, 1236, 1053, 1018, 930, 867, 840, 799, 743 cm^{-1}; NMR (CCl_4) δ 1.22 (6, s), 1.65 (3, s), 1.92 (2, m), 2.50 (2, m), 3.83 (2, s); uv_{max} (CH_3OH) 232 nm (ϵ 10 200).

Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22. Found: C, 67.63; H, 7.96.

b) By Jones Oxidation of Hydroxy Ester 16. To a solution of 127 g (0.64 mole) of **16** and 1 l. of acetone at 10–15 °C under nitrogen was added 200 ml of Jones reagent with stirring. Addition was completed in 40–45 min. Isopropyl alcohol was then added to destroy the excess Jones reagent. Most of the acetone was evaporated and water was added to the green residue. The product was extracted with ether (4 × 250 ml) and the extract was washed with water and brine and dried. Evaporation of the solvent gave 123.5 g (98%) of methyl ester **9b** of satisfactory purity.

Methyl 2-Bromoethyl-6,6-dimethyl-3-oxocyclohex-1-ene-1-carboxylate (17b). A mixture of 122 g (0.62 mol) of undistilled keto ester **9b,** 133 g of *N*-bromosuccinimide, and 700 ml of carbon tetrachloride was refluxed under nitrogen with illumination under a 125-W tungsten lamp. After 1 h, the reaction was judged complete by the floating succinimide. The cooled reaction mixture was filtered and concentrated to give 172 g (100%) of bromide **17b** as a yellow oil. This product contained 2–3% of unreacted starting material as the only major impurity by NMR analysis and was used in subsequent transformations without further purification. Pure **17b** was obtained by vacuum distillation: bp 122 °C (0.4 mm); ir (film) 1728, 1684, 1613, 1430, 1245, 1150, 1118, 1082, 1025, 982, 878, 820, 798 cm^{-1}; NMR (CCl_4) δ 1.27 (6, s), 1.93 (2, m), 2.58 (2, m), 3.92 (3, s), 4.03 (2, s); uv_{max} (CH_3OH) 230 (ϵ 8580), 245 nm (shoulder) (ϵ 7780).

Anal. Calcd for $C_{11}H_{15}O_3Br$: C, 48.01; H, 5.50. Found: C, 48.29; H, 5.52.

Methyl 1,4-Dioxo-7,7-dimethyl-4,5,6,7-tetrahydroindan-2-carboxylate (18) and Enol 19. To a solution of 92 g (1.7 mol) of sodium

methoxide in 850 ml of methanol, stirred at room temperature under nitrogen, was added 238 g (1.8 mol) of dimethyl malonate. The mixture was cooled in an ice-water bath to 5 °C and was maintained at this temperature as a solution of 116.5 g (0.42 mol) of methyl 2-bromomethyl-6,6-dimethyl-3-oxocyclohex-1-ene-1-carboxylate (**17b**) in 200 ml of methanol was added dropwise. After the addition was completed (ca. 1 h), the cooling bath was removed, and the mixture was stirred under nitrogen at room temperature for 24 h. The reaction mixture was then refluxed under nitrogen for 6 h to ensure complete reaction. After cooling in an ice bath, the reaction mixture was neutralized by dropwise addition of acetic acid. The mixture was poured into water and extracted with three portions of benzene. The combined benzene extracts were washed twice with water, twice with saturated $NaHCO_3$ solution, four times with water, and once with saturated NaCl solution. The dried benzene extracts were concentrated under reduced pressure to remove benzene and part of the excess dimethyl malonate. The partially solidified residue was crystallized from ethyl acetate to give 86.23 g (86%) of a mixture of **18** and **19** (mp 150–154 °C). The proportions of the keto and enol forms in the isolated solids varied from run to run judging by the NMR spectra, although the latter usually predominated. This solid product was used in the subsequent transformation without further purification.

A sample containing nearly all enol **19** was recrystallized from ethyl acetate to give the analytical sample: mp 156.5–158 °C; ir (KBr) 3250 (br, OH), 1690, 1658, 1604 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (6, s), 1.95 (2, m), 2.53 (2, m), 3.27 (2, s), 3.83 (3, s), 10.25 (1, bs).

Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 66.24; H, 6.79.

1,4-Dioxo-7,7-dimethyl-4,5,6,7-tetrahydro-2-indanacetic Acid (20). To a solution of 21.3 g (0.09 mol) of the diketo ester (containing varying amounts of keto and enol forms **18** and **19**) in 400 ml of tetrahydrofuran, stirred under nitrogen at room temperature, was added 26.0 g of anhydrous potassium carbonate followed by 25 ml of methyl bromoacetate. The mixture was stirred under nitrogen for 48–84 h as required for the reaction to be complete. The extent of reaction was determined by thin layer chromatography (Al_2O_3 plates; $CHCl_3$–acetone, 4:1). The mixture was diluted with water and extracted with three portions of ether. The combined ether extracts were washed successively with water, saturated $NaHCO_3$ solution, and water. After concentrating under reduced pressure, the residue (29.1 g) was dissolved in a solution of 350 ml of glacial acetic acid and 350 ml of 6 N HCl. The solution was heated to boiling under nitrogen with slow distillation for 3 h. The dark reaction mixture was cooled, diluted with water, and extracted with ethyl acetate. The combined extracts were washed with water and saturated NaCl solution. The dried extracts were concentrated, and the residue was crystallized from benzene to give 12.75 g (mp 136–137 °C) of diketo acid **20.** A second crop of 2.63 g (mp 135–136 °C) was obtained from the mother liquors for a total yield of 72%. Recrystallization from benzene afforded pure material: mp 136.5–137 °C; ir (KBr) 3600–2800, 1737, 1695, 1657 cm^{-1}; uv_{max} (95% C_2H_5OH) 260 nm (ϵ 13 300); NMR ($CDCl_3$) δ 1.33 (6, s), 1.84–2.2 (2, m), 2.4–3.3 (7, m), 10.6 (1, brs).

Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 66.37; H, 6.85.

Treatment of the acid **20** with ethereal diazomethane gave the corresponding methyl ester as an oil: ir (film) 1735, 1705, 1685 cm^{-1}.

Reduction of Diketo Acid 20. To a solution of 3.54 g (15 mmol) of diketo acid **20** in 200 ml of methylene chloride, stirred under nitrogen at −70° or less (solid CO_2–acetone cooling bath), was added 35 ml of a 1.5 M solution of diisobutylaluminum hydride in toluene (52.5 mmol) dropwise so as to maintain a temperature of −70° or less. After stirring for 2 h in the cold, excess hydride was destroyed by dropwise addition of 100 ml of 20% sulfuric acid (Caution! Heat and frothing!). The mixture was diluted with water and extracted with methylene chloride. The combined extracts were washed with water and saturated NaCl solution and dried. Concentration afforded 3.25 g of crude product which was chromatographed on silica gel (ethyl acetate–benzene gradient) to give 2.10 g (63%) of a mixture of hydroxy lactones **21** and **22** (ratio ca. 1:1). This mixture was crystallized from benzene–hexane giving 0.87 g of 1α,4α-dihydroxy-7,7-dimethyl-4,5,6,7-tetrahydroindan-2α-acetic acid γ-lactone (**21**): mp 143–144 °C; NMR ($CDCl_3$) δ 1.08 (3, s), 1.14 (3, s), 1.4–3.0 (9, complex), 4.12 (1, t, J = 5 Hz), 5.48 (1, d, J = 6.8 Hz); ir ($CHCl_3$) 3600 and 3480 (OH), 1764 cm^{-1} (lactone C=O).

Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.30; H, 8.15.

The stereochemistry of **21** was established by its conversion to (±)-strigol.

The filtrate was chromatographed on neutral alumina column (2 × 25 cm). The column was eluted with a gradient system consisting of 500 ml of ethyl acetate–benzene (5:95) in the mixing chamber and 500 ml of ethyl acetate–benzene (50:50) in the reservoir chamber. Concentration of fractions gave 1α,4β-dihydroxy-7,7-dimethyl-4,5,6,7-tetrahydroindan-2α-acetic acid γ-lactone (**22**) as an oil: calculated mass for $C_{13}H_{18}O_3$ 222.1256, found *m/e* 222.1258; ir ($CHCl_3$) 3600 (OH), 1760 (lactone C=O); NMR ($CDCl_3$) δ 1.09 (3, s), 1.13 (3, s), 1.4–9.0 (9, complex), 4.18 (1, t, J = 4.9 Hz), 5.49 ppm (1, d, J = 5.4 Hz).

Formylation of Hydroxylactone 21. Methyl formate (20.0 ml) was added to a stirred mixture of sodium hydride (7.6 g, 50% suspension in oil) and hydroxy lactone **21** (5.5 g) in 200 ml of dry ether under nitrogen at ambient temperature. After 24 h of stirring, another 5.0 ml of methyl formate was added, and stirring was continued for another 8 h. The mixture was diluted cautiously with 10% $NaHCO_3$ solution and extracted with ethyl acetate. The aqueous layer was acidified with 10% HCl solution and extracted with ethyl acetate. The acidic extract was washed with water and saturated NaCl solution and concentrated under reduced pressure to give 5.8 g (92.8%) of hydroxymethylene lactone **2.** Before concentrating the ethyl acetate extract small amounts of hydroxymethylene lactone crystallized which was difficult to redissolve: mp 205–207 °C dec; ir (KBr pellet) 3300 (OH), 1720 and 1675 cm^{-1} (carbonyls); NMR (Me_2SO-d_6) δ 7.45 (1, d, J = 2.0 Hz).

Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.02; H, 7.05.

Formylation of Hydroxy Lactone 22. In the same manner as described for **21,** hydroxy lactone **22** (166 mg) was treated with sodium hydride and methyl formate in ether to give 133 mg of crude hydroxymethylene lactone **3,** which was used in the subsequent transformation without purification.

Alkylation of 2 with Bromobutenolide 4. Bromobutenolide **4** (175 mg) was added to a mixture of crude hydroxymethylene lactone **2** (137 mg), anhydrous K_2CO_3 (154 mg), and hexamethylphosphoric triamide (5 ml) with stirring under nitrogen at ambient temperature. After 10 h of stirring, another 100 mg of bromobutenolide **4** was added, and stirring was continued for another 10 h. The reaction mixture was then diluted with water and extracted with ethyl acetate. The extract was washed with water and saturated NaCl solution and dried. Solvent was removed under reduced pressure giving 222 mg of crude product. TLC showed the presence of excess unreacted **4** and two additional components. This mixture was cleanly separated by chromatography over a silica gel column (2 × 20 cm). The column was eluted with a gradient system consisting of 300 ml of benzene in the mixing chamber and 300 ml of benzene–ethyl acetate (50:50) in the reservoir chamber. The slower moving component, R_f = 0.2 ($CHCl_3$–acetone, 4:1), was crystallized from ethyl acetate–hexane giving 55 mg (27%) of (±)-strigol (**23**): mp 202–205 °C dec; calculated mass for $C_{19}H_{22}O_6$ 346.14154, found *m/e* 346.14202; the ir, uv, NMR, and mass spectra were identical with those for natural (+)-strigol.[12] NMR: δ 1.10 (3, s), 1.18 (3, s), 1.3–2.18 (4, complex), 2.02 (3, t, J = 1.5 Hz), 2.70 (2, br d, J = 6.4 Hz), 3.63 (1, m), 4.16 (1, br), 5.50 (1, dq, J = 7.8, ≈1.5 Hz), 6.13 (1, pentet, J = 1.5 Hz), 6.92 (1, pentet, J = 1.5 Hz), 7.45 (1, d, J = 2.4 Hz).

The faster moving component, R_f = 0.32 ($CHCl_3$–acetone, 4:1), was crystallized from ethyl acetate–hexane giving 40 mg (18%) of (±)-4′-epistrigol (**24**): mp 178–180 °C; calculated mass for $C_{19}H_{22}O_6$ 346.14154, found *m/e* 346.14170; ir, uv, and mass spectra were identical with those for natural (+)-strigol.[12] NMR: δ 1.10 (3, s), 1.17 (3, s), 1.3–2.16 (4, complex), 2.02 (3, t, J = 1.5 Hz), 2.69 (2, br d, J = 6.3 Hz), 3.63 (1, m), 4.09 (1, br), 5.59 (1, dq, J = 7.8, ≈1.5 Hz), 6.15 (1, pentet, J = 1.5 Hz), 6.92 (1, pentet, J = 1.5 Hz), 7.44 (1, d, J = 2.4 Hz).

Alkylation of 3 with Bromobutenolide 4. Bromobutenolide **4** (152 mg) was added to a mixture of hydroxymethylene lactone **3** (133 mg, 0.532 mmol), anhydrous K_2CO_3, and 5 ml of hexamethylphosphoric triamide with stirring under nitrogen at ambient temperature. After stirring for 18 h, water was added and the mixture was extracted with ether. The ether extract was washed with water and saturated NaCl solution. The dried extract was concentrated, and the products were partially separated by preparative TLC (silica gel; $CHCl_3$–acetone, 4:1) to give (±)-4-epistrigol (**25**) and (±)-4,4′-diepistrigol (**26**). The isolated products were designated strigol isomers C and D since it was

not possible to establish their relative assignments between **25** and **26.** The less-polar of these two products, C, R_f = 0.34, was crystallized from ethyl acetate–hexane: mp 182–184 °C; calculated mass for $C_{19}H_{22}O_6$ 346.14154, found *m/e* 346.14163; uv, ir, and mass spectra were identical with those for natural (+)-strigol; NMR was identical with that of (±)-4′-epistrigol, NMR: δ 1.10 (3, s), 1.14 (3, s), 1.24–2.12 (4, complex), 2.02 (3, t, *J* = 1.5 Hz), 2.34 (1, dt, *J* = 16.8, ≈2 Hz), 3.06 (1, dd, *J* = 16.8, 9.0 Mz), 3.63 (1, m), 4.16 (1, br t, *J* = 4.9 Hz), 5.55 (1, dd, *J* = 7.8, 1.5 Hz), 6.17 (1, pentet, *J* = 1.5 Hz), 6.96 (1, pentet, *J* = 1.5 Hz), 7.46 (1, d, *J* = 2.7 Hz).

The more polar product, D, R_f = 0.30, was obtained as a gum; calculated mass for $C_{19}H_{22}O_6$ 346.14154, found *m/e* 346.14163; uv, ir, and mass spectra were identical with those for natural (+)-strigol; NMR was identical to that for (+)-strigol, NMR: δ 1.10 (3, s), 1.14 (3, s), 1.26–2.13 (4, complex), 2.02 (3; t, *J* = 1.5 Hz), 2.35 (1, dt, *J* = 16.8, ≈2 Hz), 3.09 (1, dd, *J* = 16.6, 9.3 Hz), 3.64 (1, m), 4.16 (1, br t, *J* = 5.1 Hz), 5.54 (1, dd, *J* = 7.8, ≈2 Hz), 6.18 (1, pentet, *J* = 1.5 Hz), 6.95 (1, pentet, *J* = 1.5 Hz), 7.45 (1, d, *J* = 2.4 Hz).

3β-Acetoxyandrost-5-ene-17β-carboxylic Acid Chloride (27). A solution of 2.02 g of 3β-acetoxyandrost-5-ene-17β-carboxylic acid in 10 ml of thionyl chloride was stirred at ambient temperature for 4 h. Excess thionyl chloride was distilled off in vacuo leaving the acid chloride **27** as a pale yellow crystalline solid which was used with purification in the subsequent step.

(+)-1α-((+)-3β-Acetoxyandrost-5-ene-17β-carbonyloxy)-4α-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydroindan-2α-acetic Acid γ-Lactone (28). The crude acid chloride **27**, prepared from 2.02 g of 3β-acetoxyandrost-5-ene-17β-carboxylic acid, was dissolved in 10 ml of pyridine and 5 ml of benzene and cooled in an ice bath. (±)-Hydroxy lactone **21** (1.17 g) was dissolved in 5 ml of pyridine and added to the cold acid chloride solution. The mixture was stirred at ambient temperature for 24 h and then poured into 50 ml of ice-cold 6 N HCl solution. The mixture was extracted with $CHCl_3$, and the extracts were washed with water and saturated NaCl solution. The dried extracts were concentrated, and the residue was triturated with ethyl acetate and filtered to remove insoluble material. Concentration afforded 2.54 g of a mixture of diastereomers **28a** and **28b.**

Separation of Diastereomers 28a and 28b. The diastereomers **28a** and **28b** were separated by preparative thin layer chromatography on silicic acid preparative plates using ethyl acetate–benzene (1:9) as the solvent system. Each plate was run six times and gave only partial separation of the two diastereomers. A mixture (2.54 g) of **28a** and **28b** was chromatographed on 16 preparative plates and after six developments using the above solvent system, the zones containing the diastereomers were cut into ten 4-mm bands. Purity of each fraction was checked by thin layer chromatography by running each plate four times in the same solvent system. The unresolved mixture of **28a** and **28b** (1.3 g) was recovered and further chromatographed on ten additional preparative plates. Once again the unresolved mixture (829 mg) obtained from the second run was chromatographed on eight or more preparative plates. After preparative chromatography on a total of 34 plates, the following five fractions were obtained: (1) isomer **28a**, 303 mg; (2) isomer **28a** containing a trace of **28b**, 292 mg; (3) unresolved mixture, 386 mg; (4) **28b** containing a trace of **28a**, 216 mg; (5) pure **28b**, 671 mg. Total recovery 1.87 g (74%). The slower-moving isomer, **28b** (671 mg), was crystallized twice from ethyl acetate–pentane affording 353 mg of pure **28b**, mp 196–197 °C; $[\alpha]^{25}D$ + 2.52° (*c* 0.8, $CHCl_3$); $[\alpha]^{25}D$ −2.85° (*c* 0.9, MeOH). The faster-moving isomer, **28a** (303 mg), was similarly crystallized from ethyl acetate–pentane, yielding 186 mg of pure **28a**, mp 214–216 °C; $[\alpha]^{25}D$ −73.1° (*c* 0.8, $CHCl_3$); $[\alpha]^{25}D$ −59.1° (*c* 0.8, MeOH).

Hydrolysis of 28b. Ester **28b** (570 mg) was heated under reflux for 12 h in 100 ml of 10% methanolic potassium hydroxide. After addition of 20 ml of water, the methanol was removed on a rotary evaporator. The reaction mixture was then acidified with 6 N HCl and extracted with four 50 ml portions of ethyl acetate. The combined ethyl acetate layer was washed with water and saturated NaCl solution, dried over sodium sulfate, and evaporated to dryness to yield 545 mg of solid residue. To separate the hydroxy lactone **21** from 3β-hydroxyetienic acid, the residue was dissolved in ethyl acetate and passed over a neutral alumina (grade III) (2 × 20 cm). The hydroxy lactone was eluted from the column with 400 ml of ethyl acetate; after evaporation of the solvent, 187 mg of **21** was obtained. This lactone was crystallized from ethyl acetate–pentane to yield a sample, mp 102–104 °C; $[\alpha]^{25}D$ −8.61° (*c* 1.4, $CHCl_3$).

Hydrolysis of 28a. The ester **28a** (180 mg) was hydrolyzed and isolated in a similar fashion giving 52 mg of (+)-hydroxy lactone **21,** mp 103–104 °C; $[\alpha]^{25}D$ +8.28° (*c* 1.3, $CHCl_3$).

Formylation of (+)-Hydroxy Lactone 21. Methyl formate (2 ml) was added to a stirred mixture of sodium hydride (100 mg, 50% suspension in oil) and (+)-hydroxy lactone **21** (82 mg) in 10 ml of dry ether under nitrogen at ambient temperature. After 24 h of stirring, the mixture was cautiously diluted with 10% aqueous sodium bicarbonate solution and extracted with ethyl acetate. The aqueous layer was acidified with 10% HCl and extracted with ethyl acetate. The combined ethyl acetate extract was washed successively with water and saturated NaCl and evaporated to dryness to yield 90 mg of the product, which was used in the subsequent alkylation.

(+)-Strigol via Alkylation of 2, Derived from (+)-Hydroxy Lactone with Bromobutenolide 4. Bromobutenolide **4** (100 mg) was added to a mixture of hydroxymethylene lactone **2** (90 mg), anhydrous K_2CO_3 (100 mg), and hexamethylphosphoric triamide (2 ml) with stirring under nitrogen. After stirring the reaction mixture at 25° for 18 h, another 100 mg of **4** was added and stirring was continued for another 18 h. The reaction mixture was then diluted with water and extracted with ethyl acetate. The combined ethyl acetate extract was washed with water and saturated NaCl, dried over sodium sulfate, and concentrated to yield 82 mg of residue. This residue was dissolved in benzene and chromatographed on a silica gel column (2 × 20 cm). The column was eluted with a gradient system consisting of 300 ml of benzene in the mixing chamber and 300 ml of ethyl acetate–benzene (1:1) in the reservoir chamber. The slower moving component (+)-strigol (25 mg), R_f = 0.2 ($CHCl_3$–acetone, 4:1), was crystallized from benzene–pentane, mp 200–202 °C; $[\alpha]^{25}D$ +293.0° (*c* 0.15, $CHCl_3$); CD, $[\theta]_{229}$ +9.5 × 10^5; $[\theta]_{204}$ −6.9 × 10^5. The faster moving component, R_f = 0.32 ($CHCl_3$–acetone, 4:1) was obtained as a gum.

Formylation of (−)-Hydroxy Lactone 21. Methyl formate (2.5 ml) was added to a stirred mixture of sodium hydride (200 mg, 50% suspension in oil) and (−)-hydroxy lactone **21** (140 mg) in 10 ml of dry ether under nitrogen at ambient temperature. After 24 h of stirring, the mixture was cautiously diluted with 10% aqueous sodium bicarbonate solution and extracted with ethyl acetate. The aqueous layer was acidified with 10% HCl and extracted with ethyl acetate. The combined ethyl acetate extract was washed successively with water and saturated NaCl and evaporated to yield 154 mg of solid residue.

(−)-Strigol via Alkylation of 2, Derived from (−)-Hydroxy Lactone with Bromobutenolide 4. Bromobutenolide **4** (150 mg) was added to a mixture of hydroxymethylene lactone **2,** anhydrous K_2CO_3 (150 mg), and hexamethylphosphoric triamide (3 ml) with stirring under nitrogen at ambient temperature. After 18 h of stirring another 150 mg of **4** was added and stirring was continued for another 24 h. The reaction mixture was then diluted with water and extracted with ethyl acetate. The combined ethyl acetate layer was washed with water and saturated NaCl, dried (Na_2SO_4), and concentrated to yield 132 mg of residue. The residue was dissolved in benzene and chromatographed over a silica gel column (2 × 22 cm). The column was eluted with a gradient system consisting of 300 ml of benzene in the mixing chamber and 300 ml of ethyl acetate–benzene (1:1) in the reservoir chamber. The slower moving component, (−)-strigol (38 mg), R_f 0.2 ($CHCl_3$–acetone, 4:1) was crystallized from benzene–pentane: mp 193–194 °C; $[\alpha]^{25}D$ −279° (*c* 0.11, $CHCl_3$); CD, $[\theta]_{229}$ −8.92 × 10^5; $[\theta]_{204}$ 6.3 × 10^5. The faster moving component (48 mg), R_f 0.32 ($CHCl_3$–acetone, 4:1) was also crystallized from benzene–pentane: mp 165–166 °C; $[\alpha]^{25}D$ +145° (*c* 0.09, $CHCl_3$); CD, $[\theta]_{242}$ −3.9 × 10^5; $[\theta]_{204}$ 10.9 × 10^5.

References and Notes

(1) For a preliminary account of a portion of this work, see J. B. Heather, R. S. D. Mittal, and C. J. Sih, *J. Am. Chem. Soc.*, **96**, 1976 (1974).
(2) Presented at the 9th IUPAC Meeting, Ottawa, Canada, June 1974.
(3) The generous financial support of the U.S. Department of Agriculture and the National Institutes of Health, AM-04874, is gratefully acknowledged.
(4) W. C. Shaw, D. R. Sheperd, E. L. Robinson, and P. F. Sand, *Weeds*, **10**, 182 (1962).
(5) A. R. Saunders, *Union S. Afr. Dep. Agric., Sci. Bull.* **No. 128**, 56 (1933).
(6) H. R. Garriss and J. C. Wells, *Plant Dis. Rep.*, **40**, 837 (1956).
(7) P. L. Botha, *Koers*, **26**, 65 (1958).
(8) J. E. Dale and G. H. Egley, *Weed Sci.*, **19**, 678 (1972).
(9) R. Brown, *Handbuch Pflanzenphysiol.*, **16**, 925 (1965).
(10) C. E. Cook, L. P. Whichard, B. Turner, M. E. Wall, and G. E. Egley, *Science*, **154**, 1189 (1966).
(11) The absolute stereochemistry of (+)-strigol has not yet been established, and the enantiomer represented in **1** has been arbitrarily chosen. All other

structures are racemic, unless otherwise indicated, and are illustrated by one enantiomer.
(12) C. E. Cook, L. P. Whichard, M. E. Wall, G. E. Egley, P. Coggon, P. A. Luhan, and A. T. McPhail, *J. Am. Chem. Soc.*, **94**, 6198 (1972).
(13) G. A. MacAlpine, R. A. Raphael, A. Shaw, A. W. Taylor, and H. J. Wild, *J. Chem. Soc., Chem. Commun.*, 834 (1974).
(14) A 5-g sample of (±)-strigol was prepared for the U.S. Department of Agriculture using the experimental procedure reported herein.
(15) B. Miller, H. Marguiles, T. Dradd, Jr., and R. Wayne, *Tetrahedron Lett.*, 3081, 3085 (1970).
(16) J. D. Fissekis and B. A. Markert, *J. Org. Chem.*, **31**, 2945 (1966).
(17) D. M. Burness, *J. Org. Chem.*, **21**, 102 (1956); "Organic Syntheses", Collect. Vol. 4, Wiley, New York, N.Y., 1963, p 628.
(18) F. Farina and M. V. Martin, *An. Quim.*, **67**, 315 (1971).
(19) J. P. Van der Merwe and C. F. Garbers, *J. S. Afr. Chem. Inst.*, **17**, 149 (1964).
(20) R. G. Welss and E. I. Snyder, *J. Org. Chem.*, **36**, 403 (1971). See also I. M. Downie, J. B. Holmes, and J. B. Lee, *Chem. Ind. (London)*, 900 (1966); J. Hooz and S. S. H. Gilani, *Can. J. Chem.*, **46**, 86 (1968).
(21) J. M. Cassady and G. A. Howie, *J. Chem. Soc., Chem. Commun.*, 512 (1974).
(22) L. Colombi, A. Bosshard, H. Schinz, and C. F. Seidel, *Helv. Chim. Acta*, **34**, 265 (1951); H. B. Henbest, B. L. Shaw, and G. Woods, *J. Chem. Soc.*, 1154 (1952).
(23) R. N. Gedye, P. C. Arora, and K. Deck, *Can. J. Chem.*, **49**, 1764 (1971).
(24) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).
(25) J. L. Marshall, K. C. Erickson, and T. K. Folsom, *Tetrahedron Lett.*, 4011 (1970).
(26) G. Wendt, *Chem. Ber.*, **74**, 1242 (1941).
(27) We thank Dr. C. E. Cook for kindly providing us with a small sample of natural strigol and copies of its ir, NMR, and mass spectra.
(28) We are grateful to Dr. D. E. Moreland of North Carolina State University, Raleigh, N.C., for these biological data.
(29) J. Dillon and K. Nakanishi, *J. Am. Chem. Soc.*, **96**, 4055 (1974).
(30) A. Horeau, *Tetrahedron Lett.*, 506 (1961).
(31) Ultraviolet (uv) spectra were determined on a Cary 15 recording spectrophotometer. Melting points, determined on a Thomas-Hoover capillary melting point apparatus, are uncorrected. Infrared (ir) spectra were taken on a Perkin-Elmer 257 grating infrared spectrophotometer. Nuclear magnetic resonance (NMR) spectra were determined on a Varian A-60A spectrometer or a Bruker 90 spectrometer. NMR shifts are expressed in parts per million downfield from internal tetramethylsilane ($\delta = 0$). Circular dichroism (CD) curves were taken in a 5-mm cell at 25° using the 6002 attachment to a Cary 60 spectropolarimeter. The dynode voltage was never allowed to exceed 0.35 V, and the signal to noise ratio was always in excess of 50:1. Mass spectra were recorded using an AEI MS-9 double focusing mass spectrometer at an ionizing voltage of 70 eV. Silica gel (MN-Kieselgel, 70–270 mesh ASTM) or Woelm neutral alumina (activity grade III) was used for column chromatography. For thin layer chromatography (TLC) 0.25-mm silica gel F-254 precoated plates (EM Reagents) were used. For preparative TLC 2-mm silica gel F-254 precoated plates were used. Plates were visualized by spraying with 3% ceric sulfate in 3 N H_2SO_4 and heating to 110° for a few minutes. Extracts in organic solvents were dried over anhydrous Na_2SO_4.
(32) R. Kuhn and I. Löw, *Chem. Ber.*, **74**, 219 (1941).
(33) L. Ruzicka and W. Brugger, *J. Prakt. Chem.*, **158**, 125 (1941).

The Chemistry of the Euphorbiaceae. A New Diterpene from *Croton californicus*

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Abstract: The major diterpenic component of the medicinal plant *Croton californicus* has been isolated and the structure determined by a combination of spectral and x-ray crystallographic studies. Methyl barbascoate (**1**) crystallizes in the space group $P2_1$ (No. 4, C_2^2) with two molecules in the unit cell. Cell constants at −164 °C are $a = 9.224$ (6), $b = 10.049$ (7), $c = 10.390$ (7) Å, and $\beta = 112.44$ (4)°. The structure was refined to $R(F) = 0.048$ and $R_w(F) = 0.058$, with the goodness of fit 1.05. The absolute stereochemistry was determined by circular dicroism comparison with (−)-methyl hardwickiate. Methyl barbascoate is a member of the growing class of trans clerodanes.

The spurge family (Euphorbiaceae) includes some 8000 species which occur in tropical and temperate regions all over the world. The largest genus of the spurge family is that of *Croton*. Many of them are odorous and contain a milky juice which is more or less poisonous.[2] The commercially available Oil of Croton derives from an Asiatic species *Croton tiglium L.* and is the source of phorbol esters, whose cocarcinogenic properties have been the object of numerous research efforts.[3,4]

As a part of a general research program on the chemistry of terpenoids from the Euphorbiaceae, we have investigated an American species, *Croton californicus*.[5] This pale olive-green perennial herb is common in sandy areas of the Mohave Desert. Indians made a hot poultice of its powdered leaves as a pain reliever for rheumatism.

The constituents of the ethanol extract of *C. californicus* can be directly compared with commercial Croton Oil by high resolution gas chromatography[6] (Figure 1). The presence of a major diterpenic component in *C. californicus* which is absent in *C. tiglium* is clearly evident. The isolation and structure elucidation of this component is now described.

The ethanol extract of *C. californicus* was chromatographed on silica gel yielding the substance in question (−)-methyl barbascoate[7] (**1**) as crystals, mp 152–153 °C. This compound analyzed for $C_{21}H_{26}O_5$ and was assigned the structure depicted in **1** (without stereochemistry) on the basis of the following spectral data. The infrared spectrum of **1** has carbonyl absorptions at 1715 and 1745 cm^{-1} due to unsaturated ester and δ-lactone. Bands at 1500 and 875 cm^{-1} indicate a β-substituted furan ring which was confirmed by an ultraviolet maximum at 212 nm and NMR signals at δ 6.4 (1 H, m) and 7.4 (2 H, m). The NMR spectrum, in addition, showed the presence of two angular methyl groups at δ 1.02 and 1.27 and a methyl ester at δ 3.7. The vinyl proton appeared as a triplet ($J = 1.5$