

RECENT DEVELOPMENTS IN NATURALLY OCCURRING ERGOSTANE-TYPE STEROIDS. A REVIEW

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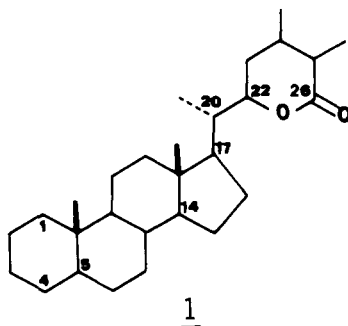
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ABSTRACT.—The withanolides are steroidal 22,26- δ -lactones built on an ergostane-type skeleton, which occur in various solanaceous plants. Several other compounds, such as 22,26- δ -lactols, 23,26- and 28,26- γ -lactones, ring D aromatic compounds, and 13,14-*seco*-derivatives, are biogenetically related to the withanolides. The work done between 1976–1980 is reviewed.

1.—Introduction.

The withanolides are a group of naturally occurring steroids built on an ergostane-type skeleton in which C-22 and C-26 are appropriately oxidized for forming a δ -lactone ring. For convenience, this basic structure was designated as the "withanolide" skeleton (1). The compounds were isolated from various solanaceous plants belonging to the genera *Withania*, *Acnistus* (*Dunalia*), *Physalis*, *Nicandra*, *Datura*, etc., where they occur mainly in the leaves.

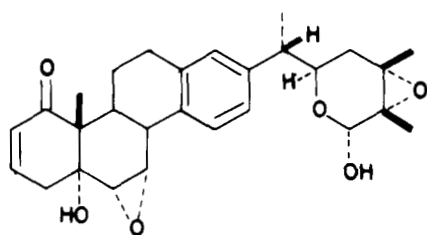
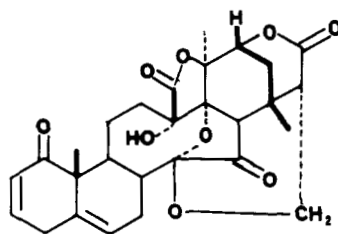
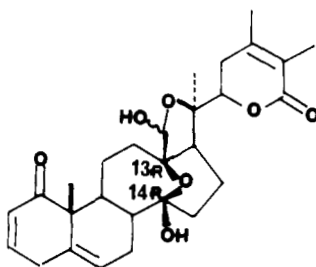


The chemistry of the withanolides and their related compounds has been reviewed (1, 2). At the time when our previous review (2) was written (1976), there was one additional group of compounds with an unmodified skeleton. These were 22,26-lactols instead of 22,26-lactones which we discussed together with the corresponding lactones. Further investigation in this area led to the discovery of several groups of compounds biogenetically related to the withanolides, but with a modified carbocyclic skeleton. Two such groups were known in 1976: a) ring D aromatic steroids, represented mainly by Nic-1 (nicandrenone) (2), and b) 13,14-*seco*-steroids, represented mainly by physalin B (3) and withaphysalin C (4).

In our previous review (2), the withanolides with an unmodified steroid skeleton (the "regular" withanolides) were subdivided according to the following structural features: a) compounds unsubstituted at C-20, e.g., withaferin A (5); b) compounds possessing a hydroxy group at C-20 (20 α F), e.g., withanolide D (6); c) compounds in which the side chain is α -oriented at C-17, e.g., withanolide E (7).

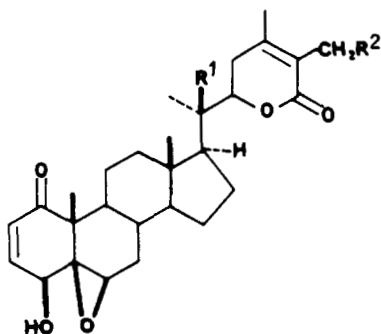
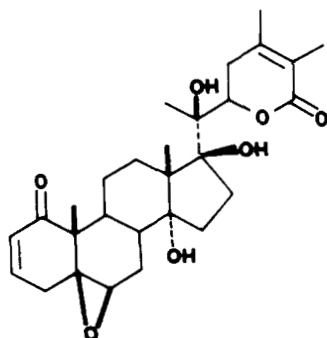
These subdivisions were important for discussing the biogenetic aspects of the problem. With very few exceptions, the plants which synthesize 20-H withanolides are unable to produce the 20-OH counterparts, and *vice versa* (3). The 17 α -alkyl withanolides are the only group of natural steroids possessing

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2 Nic-1 (nicandrenone)3 Physalin B4 Withaphysalin C

an α -oriented side chain with more than two carbon atoms; the only other natural 17α -alkyl steroids are pregnan-20-one derivatives (4).

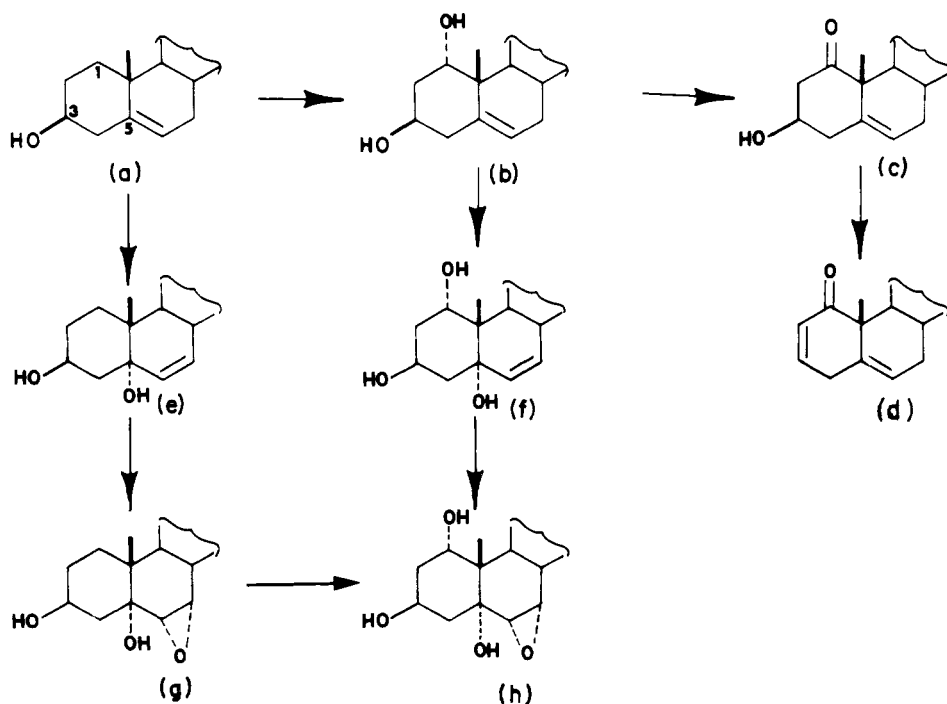
In this review, we will present the latest developments (1976-1980); a few omissions from the previous review are also covered. ^{13}C nmr spectroscopy was instrumental in the elucidation of the new structures and in the correction of several previous errors (5). For convenience, the generic name withanolides will be used not only for the ergostane-type 22,26-lactones, but also for all the biogenetically related compounds. The common features are the basic modified

5 $\text{R}^1 = \text{H}; \text{R}^2 = \text{OH}$ Withaferin A7 Withanolide E6 $\text{R}^1 = \text{OH}; \text{R}^2 = \text{H}$ Withanolide D

or unmodified ergostane skeleton and the oxidation, at various levels, of C-1, C-22, and C-26.

2.—*1 α -Hydroxywithanolides.*

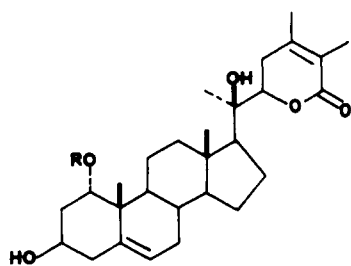
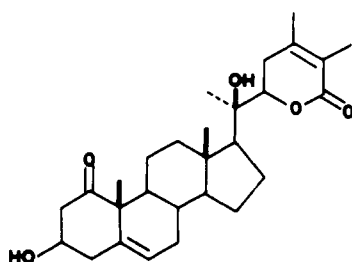
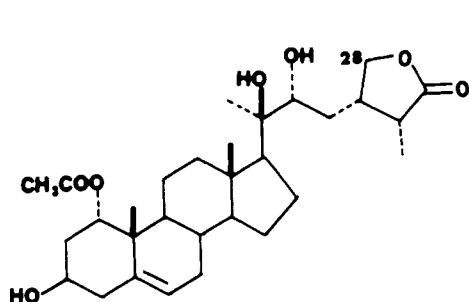
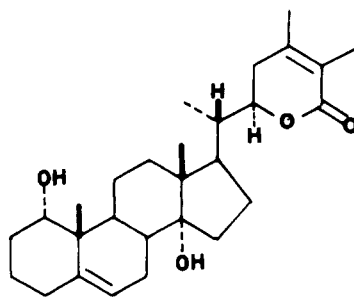
The first step in the elaboration of the substitution pattern of rings A and B in withanolides (see for instance compounds 4-7) is the hydroxylation at C-1 (see scheme 1) from the rear, less hindered side of the molecule of a 24-methylene-cholesterol (a) precursor (6). Further oxidation of such a structure (b) should lead to the corresponding 1-one (c); this would easily eliminate H₂O to give the Δ^2 -1-one system (d) which is encountered in most of the known withanolides.



Scheme 1. Hydroxylation at C-1, first step in the elaboration of the substitution of rings A and B.

The only 1α -hydroxywithanolide described in the previous review (2) was $6\alpha,7\alpha$ -epoxy- $1\alpha,3\beta,5\alpha$ -trihydroxywith-24-enolide (7) (partial structure h); it was formed, presumably, through a slightly modified pathway, as shown in scheme 1 (abfh or aegh).

Three compounds possessing partial structure b have recently been isolated and characterized. $1\alpha,3\beta,20\alpha_F$ -Trihydroxywitha-5,24-dienolide (**8a**) is a minor component of *Withania somnifera* chemotype III (8). The corresponding 1-acetate (**8b**) (physalolactone B) was isolated (9) from a variety of *Physalis peruviana* that grows near Varanasi (Uttar Pradesh, India). Compound **8a** was accompanied (10) in the plant by the corresponding 1-one (9), representing the intermediate stage towards the Δ^2 -1-one structure (scheme 1,d). Compound **8b** was accompanied in the plant by perulactone (10), possessing the same substitution in rings A and B but with a saturated side chain in which an additional hydroxy group at C-28 allowed the closure of a γ -lactone; it is the only instance encountered so far in this series where C-28 is oxidized (11).

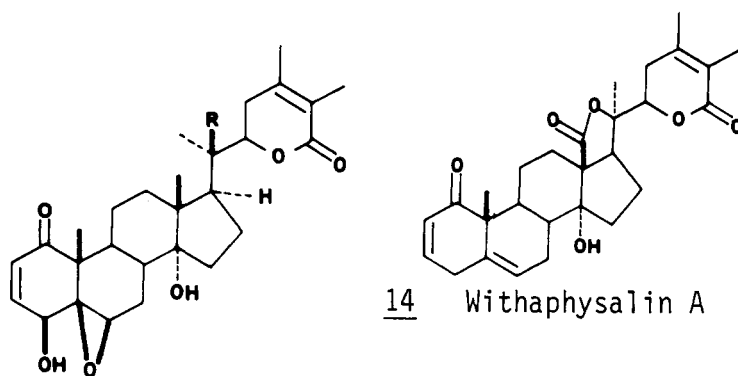
8a R = H8b R = Ac Physalolactone B910 Perulactone11

The carbocyclic moiety of perulactone was identified by cleavage of the 20,22-bond (HIO_4 or activated MnO_2) to give 1α -acetoxy- 3β -hydroxypregn-5-en-20-one. The structure of the side chain was determined by spectral analysis of perulactone itself and by identification of the γ -lactonocarboxylic acid obtained by cleavage of the 20,22-bond with CrO_3 . An additional 1α -hydroxy-withanolide ($1\alpha,14\alpha$ -dihydroxywitha-5,24-dienolide) (**11**) (**12**) was isolated from *Withania aristata* Pauq. Such a 3-deoxy structure was presumably generated by reduction of a $\Delta^{2,5}$ -1-one.

3.—14-Hydroxywithanolides.

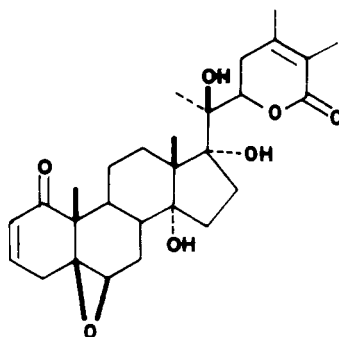
The first such compound to be discovered was 14α -hydroxy-27-desoxywithaferin A (**12**) (**13**), a minor companion of withaferin A in *Withania somnifera* chemotype I. Later, 14α -hydroxywithanolide D (**13**) (**13b**), withanolide E (**7**) (**14**) and related compounds with an α -oriented side chain, withaphysalin A (**14**) (**15**), and others were characterized.

During a re-investigation of *Withania somnifera* chemotype III, a stereoisomer of withanolide E was isolated and identified as 17 -isowithanolide E (**15**) (**10**). The compound proved to be identical with the $5\beta,6\beta$ -epoxide obtained by epoxidation with peracid of withanolide J (**17**). The structures of withanolide J and of several other related compounds (**16**–**21**) which were previously assigned (**16**) a $\Delta^{8(14)}$ structure were, therefore, re-investigated; they were all found to be 14α -hydroxywithanolides (**5**, **10**).



12 R = H; 14 α -Hydroxy-27-desoxywithaferin A

13 R = OH; 14 α -Hydroxywithanolide D

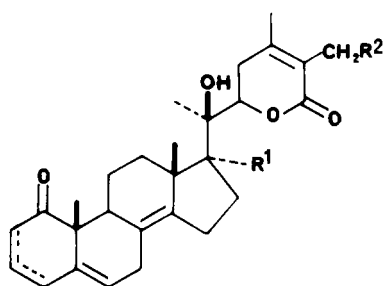


15 17-Isowithanolide E

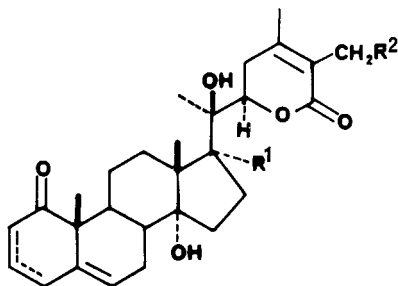
The new structures assigned to withanolides G and J are based on ^{13}C nmr, field desorption mass spectrometry, and on their behavior in the presence of trichloroacetyl isocyanate (TAI), but the structural revision of compounds (18–21) is based only on ^{13}C nmr. The first two methods were not used in the earlier investigation, (16) and the wrong results which were obtained at that time with TAI were due to the low resolution of the ^1H 60 MHz instrument. The re-investigation was performed at 270 MHz. Withanolide G (16) gave a 14,20-*bis*-trichloroacetylcarbamate (*bis*-TAC) derivative, which easily eliminated the elements of one molecule of trichloroacetylcarbamic acid to give a Δ^{14} -20-TAC-derivative. Withanolide J (17) also gave a 14,20-*bis*-TAC-derivative (a), which eliminated the 14-TAC substituent (b). As a result, the 17 α -hydroxyl became accessible to the reagent, leading slowly to the final product (c), a Δ^{14} -17,20-*bis*-TAC-derivative.

Although several other withanolides that were assigned a $\Delta^{(14)}$ -structure were not available for re-investigation, it is almost sure that they also are 14 α -hydroxy compounds [e.g., withanolide O (13b) and three yet unpublished compounds (17)]. A tetrasubstituted 8,14-double bond was assigned (18) to physalactone (23), a withanolide isolated from *Physalis alkekengi*, which grows in Turkmen SSR. The $\Delta^{(14)}$ assignment is based on uv absorption at 205 nm and on fragmentation under electron impact. A ^{13}C nmr investigation would be very useful in order to clarify whether this compound is not actually a 14 α -hydroxywithanolide. According to the ^1H nmr data, it is most probable that the side chain is α -oriented, which would make this compound a 2,3-dihydro-3-methoxy-4 β -hydroxywithanolide E.

The first 14 β -hydroxywithanolide (14 β -hydroxywithanone) (24) (19) has recently been isolated from a hybrid plant resulting from the cross polination of

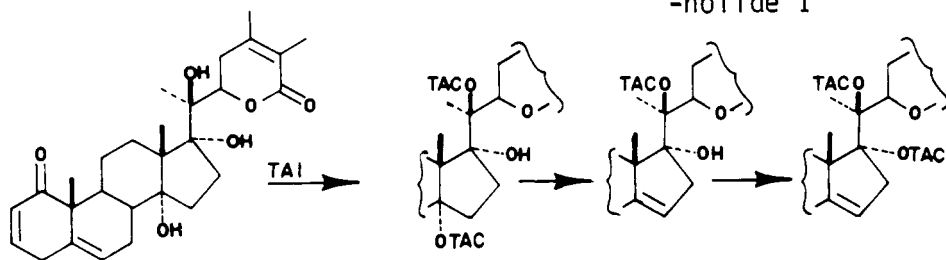
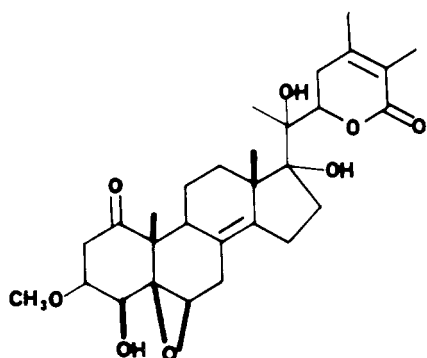
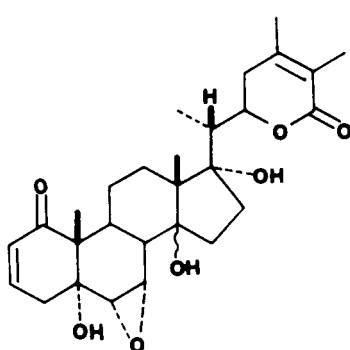


wrong structure



correct structure

- | | | |
|-----------|--|-------------------------------|
| <u>16</u> | Δ^2 , $R^1 = R^2 = H$; | Withanolide G |
| <u>17</u> | Δ^2 , $R^1 = OH$, $R^2 = H$; | Withanolide J |
| <u>18</u> | Δ^2 , $R^1 = H$, $R^2 = OH$; | Withanolide H |
| <u>19</u> | Δ^2 , $4\beta-OH$, $R^1 = R^2 = H$; | Withanolide U |
| <u>20</u> | Δ^3 , $R^1 = R^2 = H$; | Withanolide I |
| <u>21</u> | Δ^3 , $R^1 = OH$, $R^2 = H$ | Withanolide K |
| <u>22</u> | Δ^3 , $R^1 = H$, $R^2 = OH$; | 27-Hydroxywitha-
-nolide I |

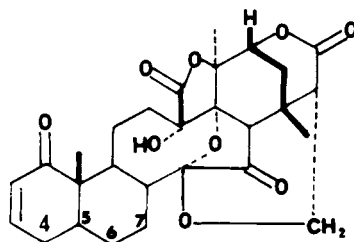
17 Withanolide Jabc23 Structure (?)
assigned to physalactone24 $14\beta-OH$
 14β -Hydroxywithanone25 $14\alpha-OH$
 14α -Hydroxywithanone

two chemotypes of *Withania somnifera*. It was accompanied in the plant by the stereoisomeric 14 α -hydroxywithanone (25). The structure of (24) was solved by crystallographic analysis. The two stereoisomers, 24 and 25, can be distinguished by ^1H nmr, the former giving the lower field signals of 7 β -H, 20-H and 13- CH_3 .

4.—New physalins.

The presence of a 14-hydroxy-group is a necessary condition for the biosynthesis of 13,14-*seco*-withanolides. Oxidative cleavage of the 13,14-bond in a 14-hydroxysteroid like (a) (scheme 2) leads to the transient formation of a 13,14-*seco*-13-hydroxy-14-oxo-derivative in a nine-membered ring system (b) which is stabilized by transformation to the 13,14-hemiketal (c), as present in withaphysalin C (4). Alternatively, when the cleavage occurs in a 14,17 α -dihydroxysteroid (d), the formation of the 14,17-hemiketal (f) is sterically favored and leads to the system (g), as present in the physalins (e.g., 3).

TABLE 1. New physalins.



Cpd	Structure	Name	Ref.	Origin	mp	$[\alpha]_D$
(26)	5 α -OH; 7 α -OH	Physalin E	(20)	<i>P. angulata</i> <i>P. lanceifolia</i> (leaves and stems)	305-307°	-83° (CHCl ₃)
(27)	5 β ,6 β -epoxy	" Fa	(21)	<i>P. angulata</i> <i>P. lanceifolia</i>	262-264°	-60.7° (CHCl ₃)
(28)	Δ^4 ; 6 β -OH	" G	(22)	<i>P. lanceifolia</i>	295-297°	+17° (acetone)
(29)	7 β -OH	" H	(20)	<i>P. angulata</i> (stems)	234-236°	
(30)	5 α -OMe; 6 β -OH	" I	(22)	<i>P. angulata</i> (stems)	?	+12° (acetone)
(31)	5 α 6 α -epoxy	" J ^b	(21)	<i>P. angulata</i> <i>P. lanceifolia</i>		
(32)	4 α ,5 α -epoxy; 6 α -OH	" K	(22)	<i>P. angulata</i> (leaves)	280-282°	

^aIdentical with the previously reported 5 β ,6 β -epoxyphysalin B (15).

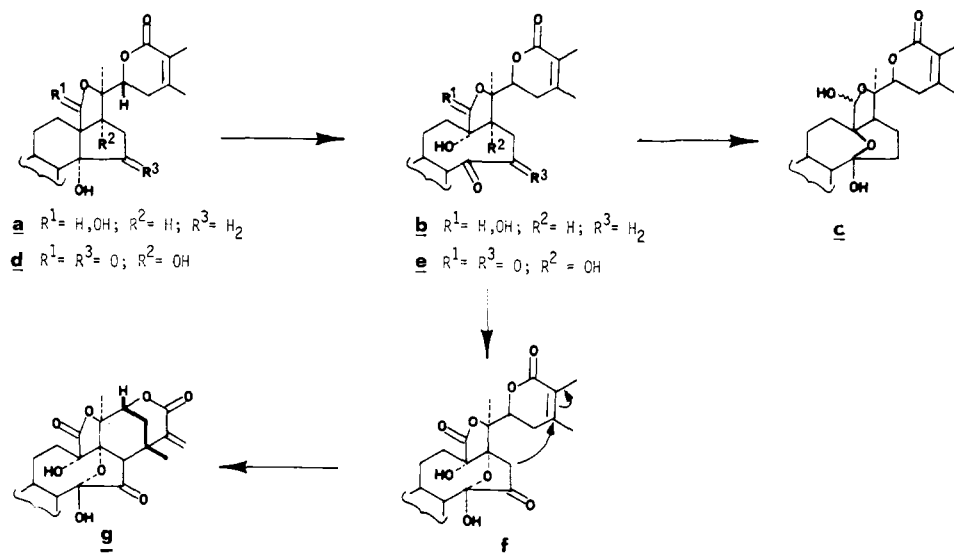
^bIdentical with the previously reported 5 α ,6 α -epoxyphysalin B obtained by peracid epoxidation of physalin B (15).

Several new physalins were isolated from *Physalis angulata* and *P. lanceifolia* (see table 1). The differences between these compounds and physalin B (3) are in the substitution at positions 4, 5, 6, and 7.

5.—15-Hydroxywithanolides.

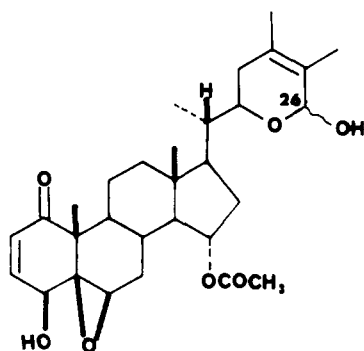
The physalins are the only known compounds related to the withanolides which are oxidized at C-15. The presence of a 15-one in a hypothetical withanolide-type precursor activates the neighboring C-16 and facilitates a Michael-type addition of the 16-anion to the unsaturated C-24; thus, a new cyclohexane ring is formed (scheme 2, f→g).

Physapubescin (33) (23), isolated from *Physalis pubescens*, was the first withanolide known to possess a 15-hydroxy group (in the form of an acetate) in an unmodified carbocyclic skeleton. In this compound, rings A and B are substituted as in withaferin A (5), while the side chain has an epoxy lactol, as in Nic-1



Scheme 2. Hypothetical pathways to physalins and withaphysalin C.

(2). The structure was solved by spectral analysis of the natural compound and of a derivative having a 15-oxo group.



33 Physapubescin

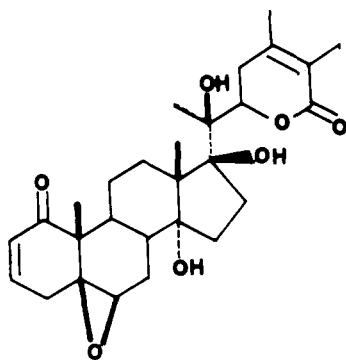
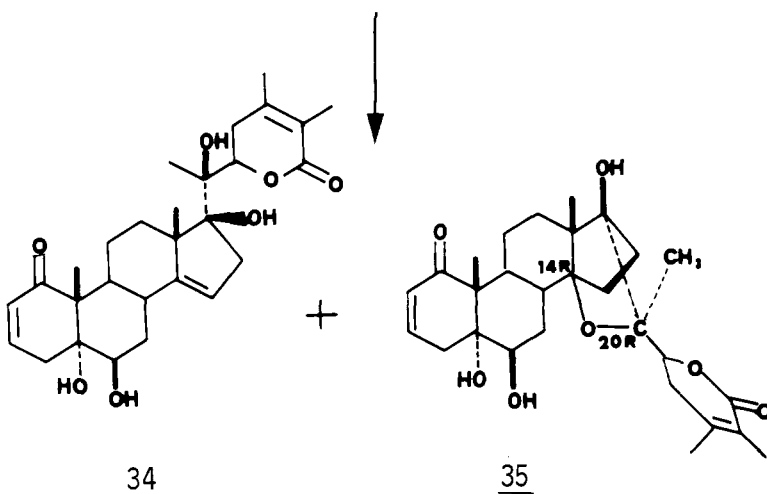
6.—16-Hydroxywithanolides.

Both 16 α - and 16 β -hydroxy steroids related to the withanolides were found in solanaceous plants. Ixocarpalactone A (24), which was isolated from *Physalis ixocarpa* Brot., possesses a 16 β -OH group, whereas nicalbin A (25), which was found in *Nicandra physaloides* var. *albiflora*, has the 16-OH group α -oriented. These compounds will be discussed in Sec. 9, dealing with side-chain modifications.

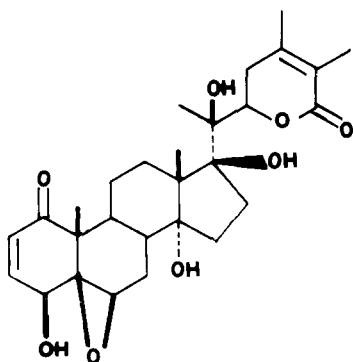
7.—17-Hydroxywithanolides.

There are three new 17 α -hydroxywithanolides, isowithanolide E (15) (10) and the two 14-hydroxywithanones (24, 25) (19). The structures of withanolides J (17) and K (21), possessing a 17 α -hydroxy-group (10), were revised (Sec. 3).

New information is available on the chemistry of 17 β -hydroxywithanolides. The acid-catalyzed dehydration of withanolide E (7) was investigated (26). Under mild conditions (a trace of 8*N* H₂SO₄ in acetone solution, for 1 h at -10°), the 14 α -OH is eliminated to give the corresponding Δ^{14} -derivative. Under more vigorous conditions (more aq. H₂SO₄, at room temperature), mixtures of the Δ^{14} -derivative (34) and the 14,20-ether (35) are formed; the 5 β ,6 β -epoxide is concomitantly transformed to the corresponding diaxial glycol.

7 Withanolide E3435

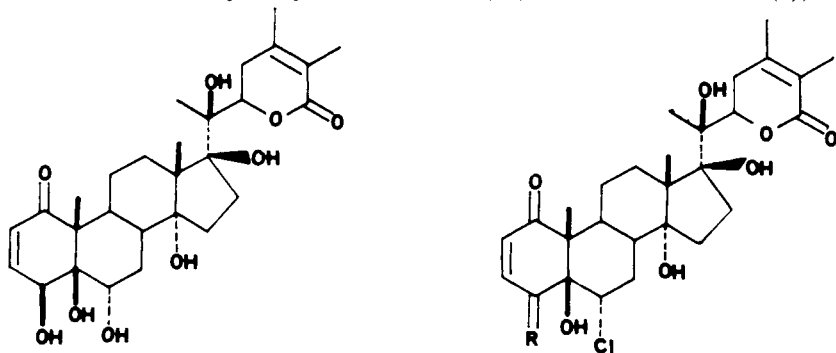
Formation of (35) can be rationalized by assuming selective protonation of the 14 α -OH with subsequent formation of a 14-carbonium ion or, alternatively, non-selective protonation of either 14- or 20-OH, to give a mixture of 14- and 20-carbonium ions. According to the first alternative, elimination of a proton from C-15 leads to (34), whereas internal nucleophilic attack by the 20-OH leads to a (14*R*,20*S*)-14,20-ether. According to the second alternative, the 14-carbonium is responsible for the formation of (34), whereas the closure of the oxide bridge is brought about by attack of the 14 α -OH on a 20-carbonium to give a (14*R*,20*S*)- or a (14*R*,20*R*)-14,20-ether. The 14*R*,20*R* configuration is supported by ¹³C nmr

36 4 β -Hydroxywithanolide E

data and by ^1H nmr [the chemical shift of 20-CH_3 (δ 1.54) in the 6,17-diacetate of (35)].

The structure of 4β -hydroxywithanolide E (36), which was established by spectral analysis (27), was confirmed (28) by chemical interrelation with withanolide E. Treatment with Al_2O_3 of the 4-tosylate of 2,3-dihydro- 4β -hydroxywithanolide E afforded withanolide E (7).

Several new compounds related to withanolide E and 4β -hydroxywithanolide E were isolated from a variety of *Physalis peruviana* that grows near Varanasi (India). Extraction of the roots afforded withaperuvin (37) (29), the product of diequatorial hydrolytic opening of (36), whereas the leaves afforded *inter alia* physalolactone (38) (30) and 4-desoxyphysalolactone (39) (29), the chlorohydrins derived from 4β -hydroxywithanolide E (36) and withanolide E (7), respectively.¹



- 37 Withaperuvin 38 R = α -H, β -OH; Physalolactone
39 R = H_2 ; 4-Desoxyphysalolactone

The diequatorial opening of the epoxide ring in (36) to give the glycol (37) and the chlorohydrin (38) could have been anticipated from previous knowledge on the behavior of 4β -hydroxy- $5\beta,6\beta$ -epoxy-steroids (31) under acidic conditions. However, the formation in nature of the diequatorial chlorohydrin 39 from withanolide E contradicts the known behavior of this compound. Treatment of withanolide E with hydrobromic acid afforded (14b) the diaxial bromohydrin only. The diaxial glycol derived from withanolide E is a natural product (withanolide S) (14b, 32).

8.—Other natural 5,6-chlorohydrins in the withanolide series.

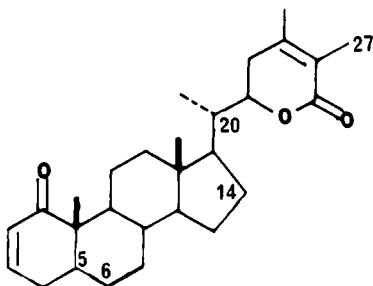
In addition to the two chlorohydrins 38 and 39, in which the side chain is α -oriented, there are several similar compounds with a normal side chain (table 2).

9.—Modifications of the side chain.

A.—*Epoxy lactols*. Oxidation at C-22 and C-26 in the 24-methylenecholesterol precursor of the withanolides at the hydroxyl ($22R$) and aldehyde level, respectively, gives an unsaturated lactol (scheme 3, a), which has not been isolated from plants, so far. Epoxidation of the 24,25-double bond in the latter gives the epoxy-lactol (b), which is characteristic for several compounds isolated from *Nicandra physaloides* (36) (Nic-1, -2, -3, -7), and from *Physalis pubescens* (23) (physapubescin, 33).

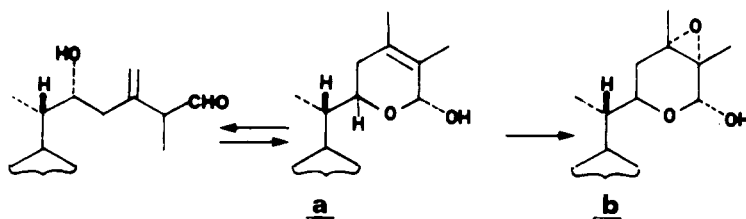
¹One of the referees wondered whether the chlorohydrin derivatives are not artifacts. The presence of compounds 38 and 39 in the leaves of *P. peruviana* was confirmed (Dr. A. Tishbee) by hplc of the crude extracts obtained by rapid extraction with methanol at room temperature. The extractions were done (Dr. A. B. Ray) with leaves collected monthly from January to May 1980. The formation of the chlorohydrins, undoubtedly by opening of the corresponding epoxides, may well be influenced by edaphic factors.

TABLE 2. 5,6-Chlorohydrins in the withanolide series.



Cpd.	Structure	Name	Source	mp	$[\alpha]_D$	Ref.
(40)	5 β ,27-di-OH; 6 α -Cl	Jaborosalactone C	<i>Jaborosa integrifolia</i>	218–20°		(33)
(41)	6 β ,27-di-OH; 5 α -Cl	Jaborosalactone E		255–58°		(33)
(42)	4 β ,5 β ,27-tri-OH; 6 α -Cl	—	<i>Withania frutescens</i>	281–84°	+68° (CHCl ₃)	(12)
(43)	4 β ,5 β ,20 α F-tri-OH; 6 α -Cl	—	Hybrid of <i>W. somnifera</i>	225–27°		(34)
(44)	4 β ,5 β ,16 α -tri-OH; 6 α -Cl	—	<i>Dunalia tubulosa</i>	247–49°	+76° (CH ₃ CN)	(35)

The preference for an axial orientation of the hemiacetal hydroxy group is well established in carbohydrate chemistry. In the withanolide-type epoxy-lactols, the 26-OH is exclusively axial (26*R*) in Nic-1 (2) and nicalbin A (45); physapubescin (33) is a 4:1 mixture of 26*R* and 26*S* stereoisomers. These determinations are based on ¹³C nmr and on high-pressure liquid chromatography; no data are available for Nic-2, -3, and -7.

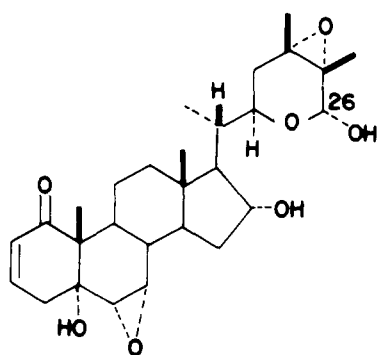


Scheme 3. Formation of epoxy-lactols

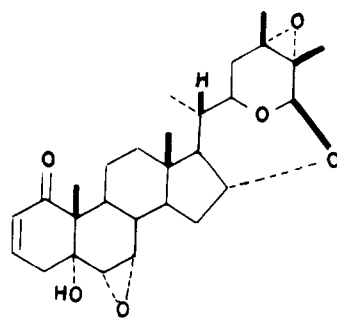
In addition, two new compounds were isolated from *Nicandra physaloides* var. *albiflora* (25): nicalbin A (45), which is the 16 α -hydroxy-derivative of Nic-3, and nicalbin B (46), which is the bicyclic acetal obtained when nucleophilic attack of the 16 α -OH on the hemiacetalic C-26 leads to the formation of a seven-membered hetero-ring. According to ¹H- and ¹³C nmr data, this ring exists in a twist-chair conformation.²

B.—*Epoxy-lactones*. The first epoxy-lactone isolated in this series was Nic-1-lactone, a minor component of *Nicandra physaloides* (37). It is one of the few natural ring D aromatic steroids. Several new epoxy-lactones possessing an unmodified steroidal carbocyclic skeleton were subsequently isolated from *Datura quercifolia* (38) (table 3).

²The structure assigned to nicalbin B was confirmed by X-ray analysis. J. D. Bordner, J. D. Thacker, and W. G. Williams, Crystal Structure Comm., 1981 (in press).

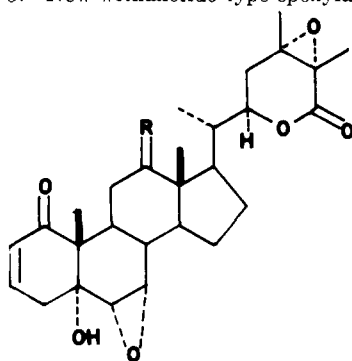


45 Nicalbin A



46 Nicalbin B

C.—*Additional oxidation at C-23*. Withanolides Q and R were the only 23-hydroxywithanolides known before 1976 (40). These compounds possess the “regular” α,β -unsaturated- δ -lactone structure, although the presence of two hydroxy groups, at C-22 ($22\beta_F$) and C-23 ($23\alpha_F$)³ would have allowed the alternative formation of a γ -lactone. Two new compounds oxidized at C-23 were isolated from *Physalis ixocarpa* (24). The major component, ixocarpalactone A (51), is the first compound related to the withanolides to possess a saturated γ -lactone. The stereochemistry of the side chain ($20\alpha_F$, $22\beta_F$, $23\beta_F$)⁴ was determined by ¹H- and ¹³C nmr of the 20,22-cyclic sulfite, 20,22-cyclic carbonate and 16,20,22-orthoacetate. The absolute configuration of the γ -lactone was determined by cd:

TABLE 3. New withanolide type epoxy lactones.^a

Cpd.	Structure	Name	mp	$[\alpha]_D$	Ref.
(47)	R=O	Daturalactone A	303-305°	+63° (CHCl ₃)	38a
(48)	R= α -OH, β -H	“ B	260-261°	0° (CHCl ₃)	38a
(49)	R= β -OH, α -H	“ C	285-286°	+73° (CHCl ₃)	38b
(50)	R=H ₂	“ D	282°		38c

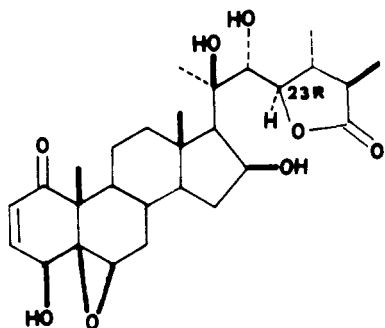
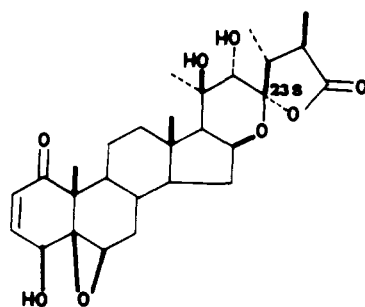
^aIn previous works (39), wrong structures were assigned to these compounds.

negative Cotton effect at 211 nm, in contrast to cyasterone which has a positive effect at 218 nm. The carbocyclic moiety was identified by cleavage of the 20,22-bond, leading to the formation of $5\beta,6\beta$ -epoxy-4 β -hydroxypregna-2,16-diene-1,20-dione.

Ixocarpalactone B (52) is a minor companion of ixocarpalactone A and has

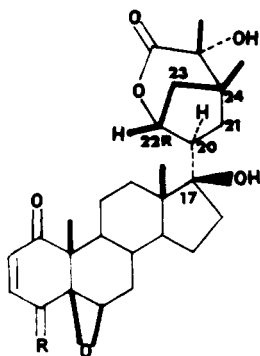
³According to the modified Fieser convention, these designations are 22β and 23β [W. R. Nes, *Adv. Lipid Res.*, **15**, 233 (1977)].

⁴ $20\alpha,22\beta,23\alpha$ -according to the modified Fieser convention.

51 Ixocarpalactone A52 Ixocarpalactone B

a spiroacetal-type structure. Its formation can be rationalized by assuming the prior oxidation of the 23-OH group to the corresponding ketone.

D.—*Additional oxidation at C-28.* Perulactone (10) is the only compound related to the withanolides in which C-28 is oxidized to the corresponding primary alcohol. Lactonization affords the 22,26- γ -lactone; the compound remains unchanged under equilibrating conditions.

53 R = α -H, β -OH Acnistin E54 R = H₂ Acnistin A

E.—*Acnistins.* A novel side chain, biogenetically related to the withanolide type is encountered in two compounds isolated from the leaves of *Acnistus ramiflorum* Miers (41). Acnistin E (53) and A (54) are derived from a precursor possessing the characteristic α,β -unsaturated δ -lactone in a 20-H, α -oriented side chain. Hydroxylation of the 21-methyl group, followed by transformation of the 21-OH into a good leaving group, must have afforded the driving force for nucleophilic attack by the π system of the 24,25-double bond, thus leading to the closure of a new cyclopentane ring. The structure of acnistin E was determined by crystallographic analysis. The difference between acnistin E and A (4-desoxyacnistin E) is in the substitution pattern of ring A. The compounds were interrelated by conversion of acnistin E into A.

10.—Analytical data and biological activity.

High-pressure liquid chromatography was applied to the separation of withanolides. In earlier work (42), withaferin A and several derivatives were separated on a 30-cm μ -Porasil column with ethyl acetate-hexane mixtures and were detected by a refractive index detector. Later Porasil A with hexane-isopropanol mixtures and a variable-wavelength detector were used to achieve good separation (43).

To compensate for the lower efficiency of this adsorbent, longer (366-cm) coiled columns were used. This procedure allowed the detection of 5 ng of withanolides. In the latest publication (44), the use of LiChrosorb SI-100 with mixtures of dichloromethane-acetonitrile-isopropanol is advocated. As in the previous work (43), the separation was monitored at 225 nm, on a variable-wavelength uv detector. However, this detector cannot be used for saturated compounds like perulactone.

In a publication dating back to 1960 (45), it was noted that Nic-1 (nicandrenone) (2) is a feeding deterrent for larvae of *Manduca sexta* (L). Several other withanolides were recently (46) found to be even stronger antifeedants when offered to larvae of *Spodoptera littoralis*.

Studies on the antitumor properties of several withanolides are being actively pursued.

LITERATURE CITED

- 1a. R. N. Tursunova, V. A. Maslennikova and N. K. Abubakirov, *Khim. Prirodn. Soedinenii*, **13**, 147 (1977), [English translation, *Chem. Nat. Compounds*, **13**, 131 (1977)].
- 1b. A. V. Kamernitskii, I. G. Reshetova and V. A. Krivoruchko, *Khim. Prirodn. Soedinenii*, **13**, 156 (1977). [English translation, *Chem. Nat. Compounds*, **13**, 138 (1977)].
2. E. Glotter, I. Kirson, D. Lavie and A. Abraham, *Bioorganic Chemistry* Vol. II. ed. by E. E. van Tamelen, Ch. 3. Academic Press, New York (1978).
- 3a. I. Kirson, E. Glotter, A. Abraham and D. Lavie, *Tetrahedron*, **26**, 2209 (1970).
- 3b. R. Hänsel, J. T. Huang and D. Rosenberg, *Arch. Pharmaz.* (Weinheim, Ger.), **308**, 653 (1975).
4. K. A. Jaeggi, E. Weiss, and R. Reichstein, *Helv. Chim. Acta*, **46**, 694 (1963); C. W. Shoppee and R. E. Lack, *J. Chem. Soc.*, 3611 (1964); H. Mitsuhashi, T. Sato, T. Nomura and I. Takemori, *Chem. Pharm. Bull.* (Japan), **12**, 981 (1964); M. Fukuoka and H. Mitsuhashi, *ibid.*, **16**, 553 (1968).
5. H. E. Gottlieb and I. Kirson, *Org. Magn. Reson.*, **16**, 20 (1981). See also S. W. Pelletier, N. V. Mody, J. Novaeki and J. Battacharyya, *J. Nat. Prod.*, **42**, 512 (1979).
6. W. J. S. Lockley, H. H. Rees and T. W. Goodwin, *Phytochemistry*, **15**, 937 (1976).
7. I. Kirson, E. Glotter, D. Lavie and A. Abraham, *J. Chem. Soc. (C)*, 2032 (1971).
8. V. Vande Velde and D. Lavie, *Israel Chem. Soc. 47th annual meeting*, September 1980, Rehovot, Israel. *Phytochemistry*, **20**, 1359 (1981).
9. A. B. Ray, A. Ali, M. Sahai, P. L. Schiff, Jr., J. E. Knapp and D. J. Slatkin, 4th Asian Symposium on Medicinal Plants and Spices. September 1980, Bangkok, Thailand, Full paper, *Chem. Ind.* (London) 62 (1981).
10. I. Kirson and H. E. Gottlieb, *J. Chem. Research* (S), 338, (M) 4275 (1980).
11. H. E. Gottlieb, I. Kirson, E. Glotter, A. B. Ray, M. Sahai and A. Ali, *J. C. S. Perkin I.*, 2700 (1980).
12. A. G. Gonzalez, J. L. Breton and J. M. Trujillo, *An. Quim.*, **70**, 64 (1974).
- 13a. E. Glotter, R. Waitman and D. Lavie, *J. Chem. Soc. (C)*, 1765 (1966).
- 13b. A. Abraham, I. Kirson, D. Lavie and E. Glotter, *Phytochemistry*, **14**, 189 (1975).
- 14a. D. Lavie, I. Kirson, E. Glotter, D. Rabinovich and Z. Shakked, *Chem. Comm.*, 877 (1972).
- 14b. E. Glotter, A. Abraham, G. Günzberg and I. Kirson, *J. C. S. Perkin I.*, 341 (1977).
15. E. Glotter, I. Kirson, A. Abraham, S. S. Subramanian and P. D. Sethi, *J. C. S. Perkin I.*, 1370 (1975).
16. E. Glotter, I. Kirson, A. Abraham and D. Lavie, *Tetrahedron*, **29**, 1353 (1973).
17. I. Kirson, unpublished results.
18. V. A. Maslennikova, R. N. Tursunova and N. K. Abubakirov, *Khim. Prirodn. Soedinenii*, **13**, 531 (1977); [English translation *Chem. Nat. Compounds*, **13**, 443 (1977)].
19. S. S. Nittala, F. Frolow and D. Lavie, *Chem. Comm.*, 178 (1981).
20. L. R. Row, N. S. Sarma, T. Matsuura and R. Nakashima, *Phytochemistry*, **17**, 1641 (1978).
21. L. R. Row, N. S. Sarma, K. S. Reddy, T. Matsuura and R. Nakashima, *Phytochemistry*, **17**, 1647 (1978).
22. L. R. Row, K. S. Reddy, N. S. Sarma, T. Matsuura and R. Nakashima, *Phytochemistry*, **19**, 1175 (1980).
23. I. Kirson, H. E. Gottlieb and E. Glotter, *J. Chem. Res.*, (S) 125, (M) 2134 (1980).
24. I. Kirson, A. Cohen, M. Greenberg, H. E. Gottlieb, E. Glotter, P. Varenne and A. Abraham, *J. Chem. Res.*, (S) 103, (M) 1178 (1979).
25. I. Kirson, H. E. Gottlieb, M. Greenberg and E. Glotter, *J. Chem. Res.*, (S) 69, (M) 1031 (1980).
26. I. Kirson, G. Günzberg, H. E. Gottlieb and E. Glotter, *J. C. S. Perkin I.*, 531 (1980).
27. I. Kirson, A. Abraham, P. D. Sethi, S. S. Subramanian and E. Glotter, *Phytochemistry*, **15**, 340 (1976).
28. K. Sakurai, H. Ishii, S. Kobayashi and T. Iwao, *Chem. Pharm. Bull.* (Japan), **24**, 1403 (1976).
29. F. Frolow, A. B. Ray, M. Sahai, E. Glotter, H. E. Gottlieb and I. Kirson, *J. C. S. Perkin I.*, 1029 (1981).
30. A. B. Ray, M. Sahai and B. C. Das, *J. Indian Chem. Soc.*, **55**, 1175 (1978).
31. S. Greenfield, E. Glotter, D. Lavie and Y. Kashman, *J. Chem. Soc. (C)*, 1460 (1967).

32. T. K. Bhattacharya, T. N. C. Vedantham, S. S. Subramanian and I. Kirson, *Indian J. Pharm. Sci.*, **40**, 177 (1978). Withanolide S was also isolated from the Varanasi variety of *P. peruviana*, A. B. Ray, personal communication.
33. R. Tschesche, M. Baumgarth and P. Welzel, *Tetrahedron*, **24**, 5169 (1968).
34. S. S. Nittala, H. E. Gottlieb and I. Kirson, 12th IUPAC Symposium on the Chemistry of Natural Products, 1980, Tenerife, Spain.
35. I. Kirson and A. Abraham, unpublished results.
36. M. J. Begley, L. Crombie, P. J. Ham and D. A. Whiting, *J. C. S. Perkin I*, 296 (1976); R. B. Bates and S. R. Morehead, *Chem. Comm.*, 125 (1974).
37. E. Glotter, I. Kirson, A. Abraham and P. Krinsky, *Phytochemistry*, **15**, 1317 (1976).
- 38a. A. K. Kalla, M. L. Raina, K. L. Dhar, M. A. Qurishi and G. Snatzke *Phytochemistry*, **18**, 637 (1979).
- 38b. M. A. Qurishi, K. L. Dhar and C. K. Atal, *Phytochemistry*, **18**, 283 (1979).
- 38c. M. A. Qurishi, A. K. Kalla and K. L. Dhar, *Phytochemistry*, **18**, 1756 (1979).
- 39a. K. L. Dhar and M. L. Raina, *Phytochemistry*, **12**, 476 (1973); K. L. Dhar and A. K. Kalla, *Phytochemistry*, **15**, 339 (1976).
- 39b. Daturalactone A was also isolated from *Datura stramonium*, V. A. Maslennikova and N. K. Abubakirov, *Khim. Prirodn. Soedinenii*, **12**, 670 (1976).
40. I. Kirson, A. Cohen and A. Abraham, *J. C. S. Perkin I*, 2136 (1975).
41. A. Usubillaga, G. de Castellano, V. Zabel and H. Watson, *Chem. Comm.*, 854 (1980).
42. M. E. Gustafson, A. W. Nicholas and J. P. Rosazza, *J. Chromatogr.*, **137**, 465 (1977).
43. I. R. Hunter, M. K. Walden, E. Heftmann, E. Glotter and I. Kirson, *J. Chromatogr.*, **170**, 437 (1977).
44. A. Tishbee and I. Kirson, *J. Chromatogr.*, **195**, 425 (1980).
45. R. T. Yamamoto and G. S. Fraenkel, *Ann. Entomol. Soc. Am.*, **53**, 503 (1960).
46. K. R. S. Ascher, N. E. Nemny, M. Eliyahu, I. Kirson, A. Abraham and E. Glotter, *Experientia*, **36**, 998 (1980).