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REVIEW ARTICLE

# THE STEROIDAL GLYCOALKALOID α-TOMATINE

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**Key Word Index**—Solanaceae, steroidal glycoalkaloid, α-tomatine, review, chemistry, biochemistry, distribution, biological activity and significance

**Abstract**—The literature relating to chemical, biochemical and biological aspects of the steroidal glycoalkaloid, α-tomatine, is reviewed The alkaloid, which can be used as a starting compound for the synthesis of steroidal hormones, is toxic to a wide range of living organisms The significance of tomatine to plants which elaborate it is discussed and some possible uses of the compound are mentioned

## INTRODUCTION

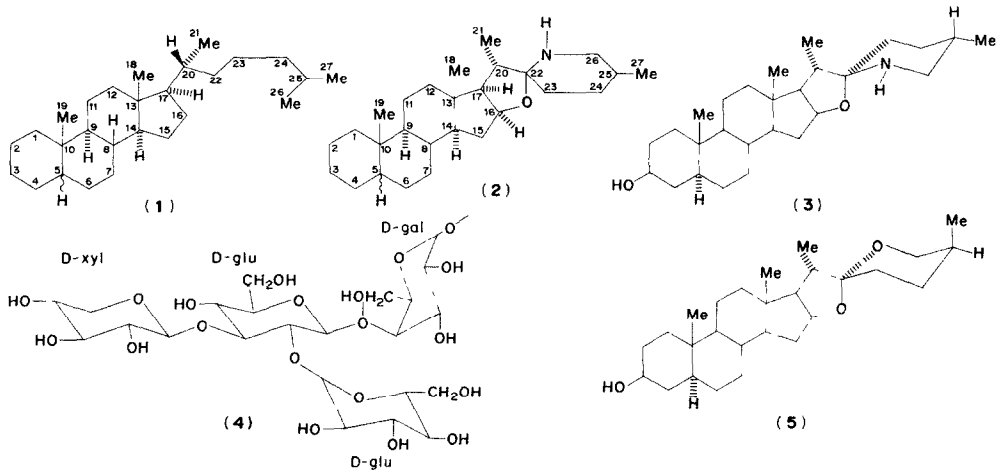
IT HAS been recognized for a number of years that the susceptibility of tomato plants to wilt caused by the fungus *Fusarium oxysporum* f *lycopersici* varies with the variety One possible reason is that different varieties produce different amounts of a compound(s) which is able to inhibit the growth of the fungus Experiments conducted by Fisher[1] and later by Gottlieb[2] to test this hypothesis revealed that expressed juice from tomato plants did, in fact, inhibit the *in vitro* growth of *F oxysporum* f *lycopersici* and further, that the degree of inhibition was proportional to the wilt resistance of the varieties tested Using a wilt resistant variety of tomato, Irving *et al*[3] confirmed this fungistatic property of expressed juice, established that the inhibition was of a chemical nature and proposed that the active principle be called "lycopersicin" A year later (1946), however, the same authors suggested[4] renaming the active principle "tomatin" since "lycopersicin" had already been used[5] as a synonym of lycopene Leaves of *Lycopersicon pimpinellifolium* were found to be a rich source of tomatin,[6] and from this material Fontaine *et al* (1948)[7] first isolated and crystallized the fungistatic agent The purified inhibitor was called "tomatine" to distinguish it from crude or partially-purified "tomatin"

## STRUCTURE AND CHEMISTRY

The first chemical studies of tomatine were conducted by Fontaine *et al*[7] who suggested the compound to be a "glycosidal alkaloid" consisting of an aglycone moiety, tomatidine, and a tetrasaccharide moiety

[1] FISHER, P L (1935) *Maryland Agr Expt Sta Bul* 374
[2] GOTTLIEB, D (1943) *Phytopathology* **33,** 1111
[3] IRVING, G W, FONTAINE, T D and DOOLITTLE, S P (1945) *Science* **102,** 9
[4] IRVING, G W, FONTAINE, T D and DOOLITTLE, S P (1946) *J Bacteriol* **52,** 601
[5] DUGGAR, B M (1913) *Wash Univ Stud* **1,** 22
[6] FONTAINE, T D, IRVING, G W and DOOLITTLE, S P (1947) *Arch Biochem* **12,** 395
[7] FONTAINE, T D, IRVING, G W, MA, R M, POOLE, J B and DOOLITTLE, S P (1948) *Arch Biochem* **18,** 467

Empirical formulae for tomatine and tomatidine have been calculated as $C_{50}H_{83}NO_{21}$ and $C_{27}H_{45}NO_2$ respectively [8,9] IR spectroscopy and other studies have indicated that tomatidine is a steroidal secondary amine with a 3$\beta$-hydroxyl group[8,10] and lacking double bonds [11] The steroid nature of the aglycone has been confirmed by degradation to 3$\beta$-acetoxy-5$\alpha$-pregn-16-en-20-one[9,12,13] and by partial synthesis from the steroidal sapogenin neotigogenin [14] The absolute configuration of tomatidine has been elucidated with the aid of techniques such as NMR spectroscopy,[15,16] X-ray diffraction [17,18] MS,[19] ORD and circular dichroism [20-22] Tomatidine (**3**) ([25*S*]-5$\alpha$-22$\beta$*N*-spirosolan-3$\beta$-ol) is a derivative of the $C_{27}$ steroidal hydrocarbon cholestane (**1**) and possesses a heterocyclic basic ring system characteristic of the spirosolanes (**2**) [23]




The tetrasaccharide moiety ($\beta$-lycotetraose) consists of two molecules of glucose and one each of galactose and xylose [24-26] The four monosaccharides form a branched structure[10,25,26] which is attached at the C-3 position of the aglycone [23] $\beta$-Lycotetraose (**4**) has been accurately characterized as *O*-$\beta$-D-glucopyranosyl-(1 → 2 glu)-*O*-$\beta$-D-xylopyranosyl-(1 → 3 glu)-*O*-$\beta$-D-glucopyranosyl-(1 → 4 gal)-$\beta$-D-galactopyranose

Partial hydrolysis of tomatine yields forms with modified sugar moieties [25] It has been

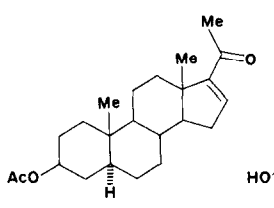
[8] FONTAINE T D ARD J S and MA R M (1951) *J Am Chem Soc* **73**, 878
[9] SATO Y KATZ A and MOSETTIG E (1951) *J Am Chem Soc* **73**, 880
[10] KUHN R LOW I and TRISCHMANN H (1956) *Angew Chem* **68**, 212
[11] PROKOSHEV S M PETROCHENKO, E I and BARANOVA V Z (1950) *Dokl Akad Nauk SSSR* **74** 339
[12] SATO Y KATZ A and MOSETTIG E (1952) *J Am Chem Soc* **74**, 538
[13] KUHN R LOW I and TRISCHMANN H (1952) *Ber* **85**, 416
[14] UHLE F C and MOORE J A (1954) *J Am Chem Soc* **76**, 6412
[15] BOLL P M and PHILIPSBORN W VON (1965) *Acta Chem Scand* **19**, 1365
[16] TOLDY L and RADICS L (1966) *Kem Kozlemen* **26**, 247
[17] HOHNE E RIPPERGER H and SCHREIBER K (1967) *Tetrahedron* **23**, 3705
[18] KENNARD O RIVA DI SANSEVERINO L and ROLLETT J S (1967) *J Chem Soc Org* **10C**, 956
[19] BARBIER M GRIEN B N WOISENHOLME W A and JENNINGS K R (1968) *Adv Mass Spectro* **4**, 89
[20] RIPPERGER H and SCHREIBER K (1965) *Tetrahedron* **21**, 407
[21] RIPPERGER H SCHREIBER K and SNATZKE G (1965) *Tetrahedron* **21**, 727
[22] SNATZKE G RIPPERGER H HORSTMANN C and SCHREIBER K (1966) *Tetrahedron* **22**, 3103
[23] SCHREIBER K (1968) in *The Alkaloids* (MANSKE R H F ed) Vol X p 1 Academic Press New York
[24] MA R M and FONTAINE T D (1950) *Arch Biochem* **27**, 461
[25] KUHN R and LOW I (1953) *Ber* **86**, 1027
[26] KUHN R LOW I and TRISCHMANN H (1957) *Ber* **90** 203

proposed[26] that the form whose sugar moiety is the tetrasaccharide be called α-tomatine and that those forms lacking xylose, lacking one glucose, and lacking xylose and one glucose be called $\beta_1$-, $\beta_2$- and γ-tomatine respectively According to this nomenclature, $\beta_1$-tomatine has been isolated from certain varieties and mutants of *Lycopersicon esculentum*[27,28] and *L pimpinellifolium*[27] (see Table 2) However, Schreiber[23] is of the opinion that such glycosides are probably either products of enzyme hydrolysis during extraction, or natural intermediates in the biosynthesis and/or degradation of α-tomatine Some physical constants of α-tomatine and its hydrolysis products are shown in Table 1

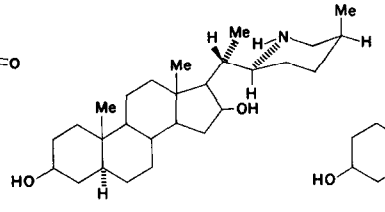
TABLE 1 PHYSICAL CONSTANTS OF α-TOMATINE AND ITS HYDROLYSIS PRODUCTS

| Compound | Formula | M p (°) | $[\alpha]_D$ (solvent) | Spectroscopic data | Ref |
|---|---|---|---|---|---|
| γ-Tomatine | $C_{50}H_{83}NO_{21}$ | 260–265 (dec ) | −29 5° (pyridine) | | 185 |
| $\beta_1$-Tomatine | $C_{45}H_{75}NO_{17}\ H_2O$ | Amorphous | −25°, −28° (pyridine) | | 26 |
| | | 265–270 (dec ) | −23 5° (pyridine) | | 28 |
| Tomatidine | $C_{27}H_{45}NO_2$ | 210 | + 6 5° ($CHCl_3$) | | 186 |
| | | 209–210 | + 5 6° ($CH_3OH$) | | 187 |
| | | 205–207 | + 5° ($CH_3OH$) | | 144 |
| | | 205–206 | + 7 6° ($CHCl_3$) | | 30 |
| | | 203–208 | + 8° ($CHCl_3$) | IR | 188 |
| | | | +25° (pyridine) | | 189 |
| | | | | IR | 8, 190 |
| | | | | ORD | 191 |
| | | | | NMR | 15 |
| | | | | MS | 192, 193 |
| | | | | $PK_b$ | 194 |

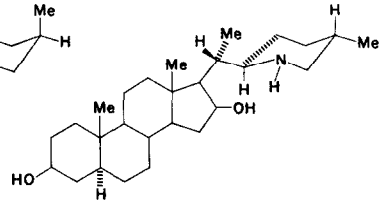
Tomatidine has been partially synthesized from neotigogenin **(5)**[14] and totally synthesized from simple total synthetically available pregnane derivatives The latter was achieved by chemical transformations of the acetyl derivative of allopregnenolone **(6)**,[29,30] by cyclization of the *N*-chloro derivative of 22,26-epimino-5α-cholestane-3β,16β-diol,[31] and by cyclization of the *N*-nitroso derivative of the latter, after irradiation with UV light[32,33]




(6)




(7)

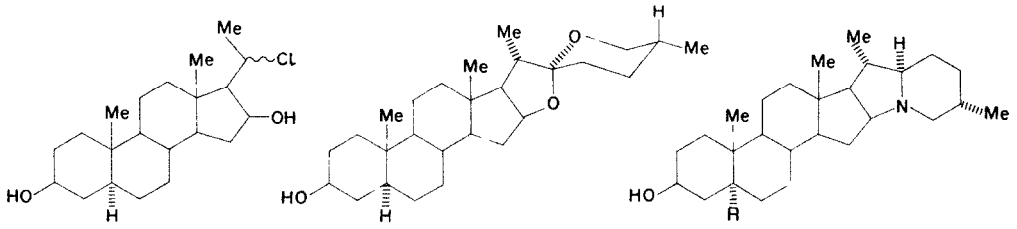



(8)

[27] SCHREIBER K HAMMER U HOF U ITHAL E and RUDOLPH, W (1961) *Tagungsber Deut Akad Landwirtschaftswiss Berlin* (27) 75
[28] SCHREIBER, K (1963) *Kulturpflanze* **11,** 502
[29] SCHREIBER, K and ADAM, G (1960) *Tetrahedron Letters* 5
[30] SCHREIBER, K and ADAM G (1963) *Ann* **666,** 155
[31] SCHREIBER, K and ADAM G (1961) *Experientia* **17,** 13
[32] ADAM, G and SCHREIBER, K (1965) *Experientia* **21,** 471
[33] ADAM, G and SCHREIBER, K (1966) *Tetrahedron* **22,** 3581

Degradation of tomatidine to 3β-acetoxy-5γ-pregn-16-en-20-one **(6)** was first reported by Sato *et al*[9 12] Since then other workers[14 34 41] have carried out this degradation in some cases using slightly different methods The 20-oxo-pregnane has also been synthesized by direct degradation of N-nitrosododecaacetyltomatine[42] Reduction of tomatidine to dihydrotomatidine A and B **(7** and **8)**[43] and UV irradiation of the *N*-chloro derivatives of these compounds yields 20-chloro-5γ-pregnane-3β,16β-diol **(9).**[33 44]

Neotigogenin and the lactone of tigogenin **(10)** have been synthesized from N-nitrosotomatidine[45] and *O* N-diacetyltomatidine[13] respectively Conversion of tomatidine and certain derivatives (e g dihydrotomatidine A) to the steroidal alkaloid demissidine **(11)** has also been reported[46,47]




Little work has been done on the microbial transformation of tomatine and its derivatives Dehydrogenation of tomatidine by *Nocardia restrictus* gave a 60% yield of 1 4-tomatidine-3-one[48] and reduction of the aglycone by *Helicostylum pulposme* yielded mainly 7α 11γ-dihydroxytomatidine (25–30%) together with smaller amounts of 7γ-hydroxytomatidine and 9γ-hydroxytomatidine[49]

The chemistry of steroidal alkaloids of the *Solanum* group, including tomatine and its derivatives has been comprehensively reviewed by Schreiber[23]

## SEPARATION AND ASSAY

In ethanol solutions, tomatine forms an insoluble 1 1 molecular complex with 3β-hydroxy steroids such as cholesterol[50] The complex is stable but can be dissociated by treatment with strong acid[50] or dimethylsulphoxide[51] This method has been widely adopted for the isolation of tomatine from crude plant extracts and can also be used to separate tomatine from its hydrolysis products since the latter are not precipitated by cholesterol

[34] Mosettig E Sato Y and Katz A (1954) *U S Pat* 2 684 365
[3] Sato Y Ikekawa N and Mosettig E (1959) *J Org Chem* **24** 893
[36] Sato Y Mosettig E and Ikekawa N (1963) *U S Pat* 3 105 068
[37] Magyar G (1961) *Tagungsber Deut Akad Landwirtschaftswiss Berlin* (27) 225
[38] Magyar G (1959) *Acta Chim Acad Sci Hung* **20**, 331
[39] Camerino B (1961) *Tagungsber Deut Akad Landwirtschaftswiss Berlin* (27) 183
[40] Schreiber K and Aurich O (1966) *Phytochemistry* **5**, 707
[41] Sato Y Ikekawa N and Mosettig E (1960) *J Org Chem* **25**, 783
[42] Schreiber K and Ripperger H (1963) *Arch Pharm* **296**, 717
[43] Adam G and Schreiber K (1969) *Z Chem* **9** 227
[44] Adam G and Schreiber K (1965) *Chem Ind* 989
[45] Sato Y Latham H G Briggs E H and Sletzi R N (1957) *J Am Chem Soc* **79** 6089
[46] Kuhn R Low I and Trischmann H (1952) *Angew Chem* **64**, 397
[47] Schreiber K and Adam G (1964) *Ber* **97**, 2368
[48] Bilic E and Socic H (1971) *Experientia* **27**, 626
[49] Sato Y and Hayakawa S (1964) *J Org Chem* **29**, 198
[50] Schulz G and Sander H (1957) *Z Physiol Chem* **308**, 122
[51] Iwadoriides C H Kitagawa I and Mosettig E (1962) *J Org Chem* **27**, 4693

In a few earlier studies tomatine was separated by PC[52-54] but more recently, TLC has proved the more successful, and hence more widely used technique A number of workers[55–59] have published articles relating to the use of this method for separation of steroidal alkaloids and sapogenins Of the compounds used for locating tomatine on TLC plates, iodine (vapour) is one of the more useful since it does not destroy the alkaloid and is readily evaporated [60] However, it has the disadvantages of being neither very specific nor sensitive Compounds which are more sensitive but do not allow recovery of the intact alkaloid are modified Dragendorff's reagent[61] and 50% $H_2SO_4$ [62] After spraying plates with the latter and heating, tomatine and tomatidine show characteristic colour changes [62,63] Separation of tomatidine has also been achieved by column chromatography,[64] GLC[65–67] and electrodialysis [68]

The methods first used for quantitative estimation of tomatine were bioassays based on the degree of growth inhibition of certain fungi (e g *F oxysporum* f *lycopersici*) in culture [7] Gravimetric methods have also been employed,[69] but, of the assays currently used, a large proportion involve spectrophotometry of a chromogen of tomatine (the alkaloid itself having no notable visible/UV absorption spectrum) Coloured products are formed by treating tomatine with strong or conc $H_2SO_4$,[70-72] a lactic acetic solution of silicotungstic acid,[73] anthrone reagent[74 75] or a molybdophosphoric acid reagent[76] (tomatine does not produce a coloured product with the Liebermann–Burchard reagent[77]) Tomatine can also be quantified by titration—of the alkaloid in non-aqueous medium,[78] and of the sugars released on acid hydrolysis [79]

## OCCURRENCE IN THE PLANT KINGDOM

Tomatine appears to be restricted in its taxonomic distribution to the family Solanaceae

[52] PETROCHENKO E I (1953) *Dokl Akad Nauk SSSR* **90,** 841
[53] TUKALO, E A (1956) *Sb Nauchn Rabot Dnepropetr Med Inst* **1,** 351
[54] TUZSON, J (1956) *Naturwissenschaften* **43,** 198
[55] BENNETT, R D and HEFTMANN, E (1962) *J Chromatog* **9,** 353
[56] SCHREIBER, K AURICH, O and OSSKE, G (1963) *J Chromatog* **12,** 63
[57] KAWASAKI, T and MIYAHARA, K (1963) *Chem Pharm Bull (Tokyo)* **11,** 1546
[58] RONSCH, H and SCHREIBER, K (1967) *J Chromatog* **30,** 149
[59] TRUHAUT, R , SCHUSTER, G and TARRADE, A M (1967) *Ann Pharm Fr* **25,** 621
[60] ADAM, G and SCHREIBER, K (1963) *Z Chem* **3,** 100
[61] CROMWELL, B T (1955) in *Modern Methods of Plant Analysis* (PAECH, K and TRACEY, M V eds ), Vol IV, p 367, Springer, Berlin
[62] HEFTMANN, E KO, S T and BENNETT, R D (1966) *J Chromatog* **21,** 490
[63] RODDICK, J G (1971) Ph D Thesis, University of Glasgow
[64] HEFTMANN, E , LIEBER, E R and BENNETT, R D (1967) *Phytochemistry* **6,** 225
[65] LLOYD, H A , FALES, H M HIGHET P F , VANDENHEUVEL W J A and WILDMAN W C (1960) *J Am Chem Soc* **82,** 3791
[66] VANDENHEUVEL, W J A , HORNING E C , SATO, Y and IKEKAWA N (1961) *J Org Chem* **26,** 628
[67] ARNESON, P A and DURBIN, R D (1967) *Phytopathology* **57,** 1358
[68] DOROSH, T P and TUKALO, E A (1961) *Farmatsevt Zh (Kiev)* **16,** 44
[69] PRABHAKAR, V S and HANDA, K L (1965) *J Proc Inst Chemists (India)* **37,** 65
[70] DIAZ, G , ZAFFARONI A , ROSENKRANTZ, G and DILRASSI, C (1952) *J Org Chem* **17,** 747
[71] WALTNS, H A , TURNER, A and WALL, M E (1954) *Anal Chem* **26,** 325
[72] RODDICK J G and BUTCHER, D N (1972) *Phytochemistry* **11,** 2019
[73] CERCOS, A P and BURACHIK, M (1955) *Rev Invest Agric (Buenos Aires)* **9,** 369
[74] SOCIC, H (1971) *Planta Med* **19,** 6
[75] LANGCAKE, P , DRYSDALE, R B and SMITH, H (1972) *Physiol Plant Path* **2,** 17
[76] FAYEZ, M B E and SALEH, A A (1969) *Fresenius' Z Anal Chem* **246,** 380
[77] SCHREIBER, K and AURICH, O (1964) *Kulturpflanze* **12,** 473
[78] GYENES, I (1964) *Tagungsber Deut Akad Landwirtschaftswiss Berlin* No 27, 177
[79] TUKALO E A (1958) *Sb Nauchn Tr Dnepropetr Med Inst* **6,** 371

and, in particular, to the genera *Solanum* and *Lycopersicon* The occurrence of tomatine and its natural derivatives within these genera is shown in Table 2 The parts of the plant from which the alkaloids have been extracted although not specified are in the majority of cases, epigeal It is interesting that in the genus *Solanum* tomatine is mostly accompanied by other steroidal glycoalkaloids, whereas in the genus *Lycopersicon* tomatine, and/or one of its derivatives is usually the only steroidal alkaloid present

TABLE 2 OCCURRENCE OF α-TOMATINE IN SOLANACEOUS PLANTS

| Species | Remarks | Ref |
|---|---|---|
| Genus *Solanum* | | |
| *S. caule* Britt | | 195 |
| *S. ceatii* Britt var *caulescens* Britt * | | 195 196 |
| *S. boerhaavii* Thell * | | 197 cf 198 |
| *S. demissum* Lindl * | | 186 |
| *S. demissum tlaxpehualcense* * | Not cited in *Index Kewensis* | 186 |
| *S. demissum utile* (= *S. demissum* Lindl var *klotzschii* Britt)* | | 186 |
| *S. depexum* Juz * | | 195 |
| *S. dulcamara* L * | | 199 200 |
| *S. kieseritzkii* C. A. Mey * | | 201 |
| *S. polyadenium* Greenm * | Also contains polysaminc† | 202 185 |
| *S. punae* Juz * | | 195 |
| *S. santonnetii* Carr | | 197 |
| *S. schickeii* Buk* | | 195 |
| *S. simplicifolium* Britt * | | 197 |
| *S. stoloniferum* Schlecht et Bouche* | | 197 |
| Genus *Lycopersicon* | | |
| *L. chesmanii* Riley | | 203 |
| *L. chilense* Dun | | 103 |
| *L. esculentum* Mill var *cerasiforme* (Dun) Alef | | 87 |
| (= *L. esculentum baccis luteo*) | | 13 203 |
| (= *L. esculentum* ssp *galeni*) | | 203 |
| (= *L. humboldtii* Dun) | | 87 204 |
| *L. esculentum* Mill var *esculentum* Mill | Also contains β tomatine | 27 39 87 138 205 208 |
| (= *L. esculentum* Mill var *succenturiatum* Prsq) | | 96 |
| (= *L. esculentum* Mill var *vulgare* Alef) | | 96 |
| *L. esculentum* Mill var *piriforme* (Dun) Alef | | 87 96 |
| (= *L. esculentum* Mill var *pumiforme* Voss) | | 13 87 96 144 208 209 |
| *L. esculentum* Mill var *grandifolium* Bailey | | 96 |
| *L. esculentum* Mill var *validum* Bailey | | 96 |
| *L. esculentum* Mill mut * | Also contains $\beta_2$ tomatine | 27 |
| *L. esculentum* Mill mut *exitis* | Contains $\beta_4$ tomatine only | 27 28 |
| *L. esculentum* Mill mut *prunoidea* | Contains $\beta_3$ tomatine only | 27 28 |
| *L. glandulosum* Mill | | 10 210 |
| *L. hirsutum* Humb et Bonpl | | 87 96 103 144 |
| *L. mexicanum* | Not cited in *Index Kewensis* | 13 |
| *L. peruvianum* (L.) Mill | | 13 96 20 |
| (= *L. peruvianum chilutatum*) | | 144 |
| (= *L. peruvianum putatum*) | | 144 |
| *L. peruvianum* (L.) Mill var *dentatum* Dun | | 103 |
| *L. peruvianum* (L.) Mill var *humifusum* Mill | | 103 203 |
| *L. peruvianum* (L.) Mill var *typicum* Mill | | 103 |
| *L. pimpinellifolium* (Jusl.) Mill * | Also contains $\beta_3$ tomatine | 7 27 87 96 138 144 205 |
| (= *L. pimpinellifolium fructo luteo*) | | 87 |
| *L. pimpinellifolium* (Jusl.) Mill var *rhexioides* (A. Voss) Lehm | | 87 |
| *L. pimpinellifolium* (Jusl.) Mill mut * | Also contains $\beta_2$ tomatine | 27 |

* Other steroidal glycoalkaloids and/or their aglycones have been isolated in addition to tomatine and/or its derivatives

† Consists of tomatidine + 2 xylose + glucose

## DISTRIBUTION IN THE PLANT

Tomatine has not been detected in dormant seeds but is probably one of the basic saponins which first appear in the radicle during the early stages of germination [80] Tomatine appears in all other organs as they develop,[81] although the amount of alkaloid varies with the organ For example, Tukalo[79] found 0 86–1 9% tomatine in leaves of tomato plants, 0 3–0 6% in stems and roots and 0 93–2 2% in fully expanded flowers However, there is also considerable variation in the reported tomatine content of particular organs, some other values for leaves being 0 46%,[82] 0 22–5 1%[83] and 1 15-2 14%,[84] for stems, 0 08%,[85] and for roots, 0 16% [86] Much of this variation appears to be attributable to such factors as the variety of plant used, the stage of development of the plant,[83,84] time of season[87] and seasonal conditions [88]

The shoot is recognized as being the main site of tomatine synthesis and accumulation, the former taking place principally in meristematic regions [81] Synthesis in the shoot appears to be independent of any direct root influence since the tomatine content of tomato shoots and its seasonal variation were not affected by grafting on to stocks of *Nicotiana*, *Datura*, *Petunia* or *Nicandra* [81] Tomatine has been isolated from callus tissue (derived from the hypocotyl of tomato seedlings) which had been maintained in culture for at least two years, but levels were very low (*ca* 0 001%) [72] The alkaloid could not be detected in root callus tissue of a similar age Tomatine has also been extracted from crown-gall diseased stem tissues of tomato [89,90]

That *de novo* synthesis of tomatine takes place in the root has been established by studies with cultured excised roots of *L pimpinellifolium*[91] and *L esculentum*[72,81] although, in the latter species, alkaloid levels per unit of dry weight were lower than in seedling roots of the same age [72] Further studies[92] suggested that this was not due to a lack of the steroid precursors, acetate, MVA or cholesterol, in the cultured root system There are some indications that the main sites of tomatine biosynthesis in the root may also be the actively growing regions [92 107]

Flowers of tomato are rich in tomatine[93] as are also, after pollination, the ovaries containing up to 1 5% alkaloid compared with *ca* 0 15% in the rest of the plant [94] Young developing fruits accumulate large amounts of tomatine, but as ripening begins alkaloid degradation occurs and concentrations decline [81] Tomatine levels of 0 087, 0 045 and 0 036% have been recorded in green, yellowish and red (ripe) tomato fruits respectively, and when ripe fruits were left on the plant for a further 2–3 days, tomatine almost completely disap-

[80] SANDER, H, HAUSER, H and HANSEL, R (1961) *Planta Med* **9**, 8
[81] SANDER, H (1956) *Planta* **47**, 374
[82] GEIGY J R AG (1958) *Swiss Pat* 328 077
[83] TOMOVA, M (1966) *Farmatsiya (Sofia)* **16**, 24
[84] TOMOVA, M (1967) *Farmatsiya (Sofia)* **17**, 24
[85] ŠICHO, V (1956) *Prumysl Potravin* **7**, 223
[86] TUKALO, E A (1958) *Sb Nauchn Tr Dnepropetr Med Inst* **6**, 377
[87] PINAR, M (1956) *Anales Real Soc Espan Fis Quim (Madrid)* **52B**, 513
[88] SIEWINSKI A MEJER, S and KOCOR, M (1957) *Przemysl Chem* **13**, 543
[89] KOVACS, B A, WAKKARY, J A, GOODFRIEND, L and ROSE, B (1964) *Science* **144**, 295
[90] CALAM, D H and CALLOW, R K (1964) *Br J Pharmacol* **22**, 486
[91] BRUSKE, H (1966) *Abhandl Deut Akad Wiss, Berlin, Kl Chem Geol Biol* **3**, 105
[92] RODDICK, J G and BUTCHER, D N (1972) *Phytochemistry* **11**, 2991
[93] TUKALO, E A (1956) *Sb Nauchn Rabot Dnepropetr Med Inst* **1**, 347
[94] HEFTMANN, E (1965) in *Plant Biochemistry* (BONNER, J and VARNER, J E eds), p 693, Academic Press, New York

peared[95] However other workers[96] have reported that although there was a decline in tomatine level during the transition from the green to the pink stage, this was followed by a slight increase as the fruits ripened fully From studies of tomatine disappearance from ripening fruits of a number of *Lycopersicon* species which show different degrees of redness, Sander[97] suggested that the isoprenoid nucleus of the degraded aglycone might be utilized in lycopene synthesis The inconsistency between reports that ripe tomato fruits are tomatine-free,[94 98] and that small amounts of tomatine are present[99 101] may therefore be a result of differences in the degree of ripeness and/or redness at the time of harvest In fact, one author[101] has claimed that the tomatine content of fruits at the stage of canning ripeness is often much higher than stated in the literature

Plants from which fruits have been removed accumulate larger amounts (up to 117%) of tomatine than fruit-bearing plants, apparently due to the loss of organs of alkaloid breakdown,[81 102] In keeping with this finding, tomatine levels in long-day vegetative plants of *Lycopersicon glandulosum* were found to be almost 5 times higher than in short-day, fruit-bearing plants[103] However, in *Lycopersicon hirsutum* and *L chilense* both of which exhibit less strong short-day characters, there was no significant difference in the tomatine content of short-day and long-day plants[103]

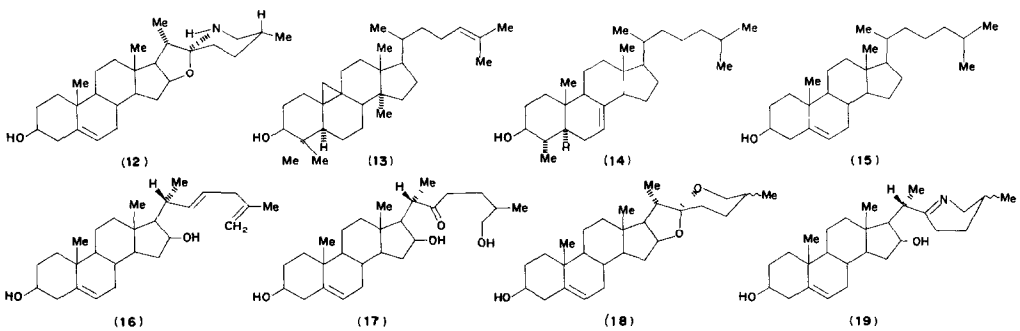
Neither the location nor the site of synthesis of tomatine within the cell are yet known and little information exists relating to transport of the alkaloid in the plant The presence of tomatine in sap exuded from decapitated plants suggests that some transport from root to shoot may occur[104] but the alkaloid has not been detected in excised root culture medium[72 81 91]

## BIOSYNTHESIS AND BIODEGRADATION

The biosynthesis of tomatidine is still not fully understood, while the processes which lead to its glycosidation are completely unknown The first studies of tomatidine biogenesis using radioactive precursors were made by Sander and Grisebach[105] who obtained labelled alkaloid from seedlings of *L pimpinellifolium* grown in the presence of acetate-[1-$^{14}$C] Solasodine (**12**) a steroidal alkaloid structurally similar to tomatidine has been isolated radioisotopically labelled, from plants of *Solanum laciniatum* ("Solanum aviculare") to which had been presented acetate-[1-$^{14}$C], acetate-[2-$^{14}$C] or mevalonate-[2-$^{14}$C][106] Degradation of this labelled solasodine revealed a distribution of the label which was consistent with the alkaloid having been synthesized via cyclization of squalene Labelled tomatine has also been isolated from cultured excised tomato roots grown in liquid medium containing acetate-[2-$^{14}$C] or MVA-[2-$^{14}$C]-lactone[107] It is now generally accepted that steroid biosynthesis in plants follows a course similar to that in animals, viz

[95] KAJDEROWICZ-JAROSINSKA, D (1965) *Acta Agr Silvestria, Ser Roln* **5,** 3
[96] PROKOSHEV, S M, PETROCHENKO E I and BARANOVA V Z (1952) *Dokl Akad Nauk SSSR* **83,** 261
[97] SANDER H (1958) *Naturwissenschaften* **45,** 59
[98] DAJANI R M KOUYOUMJIAN C and HARRISON, M (1967) *Lebanese Pharm J* **9,** 110
[99] KAJDEROWICZ-JAROSINSKA D (1965) *Advan Frontiers Plant Sci* **10,** 57
[100] KOLANKILWICZ J, MLODECKI, H and SZYMCZYK, F (1956) *Roczniki Panstwowego Zakladu Hig* **7,** 537
[101] MARNAEVA B M (1965) *Izv Vysshikh Uchebn Zavedenii Pishchevaya Tekhnol* **4,** 36
[102] SANDER H (1956) *Arch Pharm* **289,** 308
[103] SANDER H (1958) *Planta* **52,** 447
[104] KERN H (1952) *Phytopathol Z* **19,** 351
[105] SANDER H and GRISEBACH H (1958) *Z Naturforsch* **13b,** 755
[106] GUSEVA A R and PASESHNICHENKO V A (1962) *Biokhimiya* **27,** 721
[107] RODDICK J G (1974) *Phytochemistry* **13,** to be published

from acetyl-CoA, via MVA, farnesyl pyrophosphate and squalene The stages following cyclization are not completely understood but the widespread distribution in the plant kingdom of the tetracyclic triterpene, cycloartenol (**13**),[108-110] and the general absence of lanosterol, suggest that the former may be the first product of cyclization in higher plants At least consistent with this hypothesis is the finding by Ripperger *et al*[111] that cycloartenol-[26,27-$^{14}$C] is incorporated into tomatidine, with the label being localized in ring F of the alkaloid The biosynthetic pathway from cycloartenol is thought to proceed via lophenol (**14**)[23] to cholesterol (**15**) (or a closely related phytosterol), involvement of cholesterol being suggested by reports that both cholesterol-[4-$^{14}$C][64,112] and cholestanone-[4-$^{14}$C][113] are incorporated into tomatidine



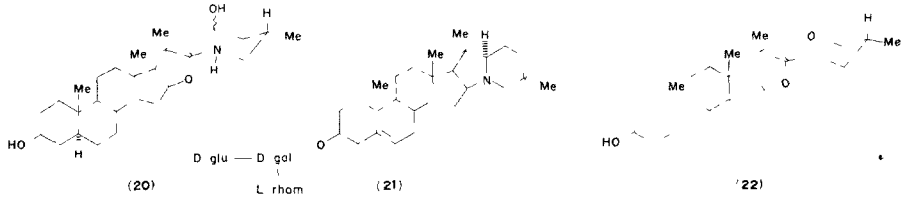

Plants which elaborate steroidal alkaloids usually contain steroidal sapogenins with the same configuration at C-25 [23] The structural similarities between these compounds render it probable that their biosynthetic pathways and metabolism are closely related and, in fact, as alkaloid levels decline in ripening fruits sapogenin levels often increase [114,115] The steroidal sapogenins present in *L. esculentum* are neotigogenin (**5**)[116] and tigogenin (**10**),[117] the latter being the sapogenin analogue of tomatidine [118] Tschesche[119] has suggested that both steroidal alkaloids and sapogenins are synthesized via the common intermediates **16** and **17**, the latter (16-dihydrokryptogenin) being known to cyclize easily to the corresponding spirostane (**18**) [120] Alkaloid biosynthesis is thought to be achieved by transamination* of **17** to the cyclic azomethine (**19**) which subsequently undergoes stereospecific cycli-

* Since the known steroidal alkaloids are derived from nitrogen-free compounds into which nitrogen is incorporated at some later stage (and not from amino acids) Hegnauer[211] has proposed that such compounds be regarded as pseudoalkaloids or 'alcaloida imperfecta' He further makes the point that in the strict sense, they would be more accurately termed "basic or nitrogenous steroids" (although these names give no indication of their biological activity) Nevertheless the term steroidal alkaloid" appears to be more widely used by both chemists and biologists than these alternatives

[108] Aexel R, Evans, S, Kelly, M and Nicholas, H J (1967) *Phytochemistry* **6,** 511
[109] Benveniste, P, Hirth L and Ourisson G (1964) *Compt Rend* **258,** 5515
[110] Ehrhardt J D Hirth L and Ourisson G (1965) *Compt Rend* **260,** 5931
[111] Ripperger H Moritz, W and Schreiber K (1971) *Phytochemistry* **10,** 2699
[112] Tschesche, R and Hulpke, H (1966) *Z Naturforsch* **21b,** 9
[113] Tschesche, R and Fritz, R (1970) *Z Naturforsch* **25b,** 590
[114] Sander, H (1963) *Planta Med* **11,** 303
[115] Willuhn, G (1967) *Planta Med* **15,** 58
[116] Sander, H (1961) *Z Naturforsch* **16b,** 144
[117] Fayez, M B E and Saleh, A A Personal communication to K Schreiber quoted in ref 23
[118] Kuhn, R, Low, I and Trischmann, H (1953) *Ber* **86,** 372
[119] Tschesche, R (1955) *Fortschr Chem Org Naturstoffe* **12,** 131
[120] Hirschmann, H and Hirschmann, F B (1958) *Tetrahedron* **3,** 243

zation to yield the spirosolane (**2**)[23] The origin of the nitrogen, however still awaits elucidation

That tomatine disappearance during fruit ripening is due to actual degradation of the alkaloid has been established by following the fate of authentic tomatine injected into isolated fruits[81] It has been proposed[121] that degradation begins at the aglycone with separation of the nitrogen-containing group preceding hydrolytic changes in the carbohydrate moiety and also[122] that *N*-acylated derivatives may be early intermediates in tomatine breakdown Other compounds isolated from *L. pimpinellifolium* and thought to be possible degradation products of tomatine are pimpinellidine (which may be 23-hydroxytomatidine [**20**]) and 3β-hydroxy-5α-pregn-16-en-20-one (the deacetyl derivative of **6**)[40] Further evidence that the latter compound is a product of tomatine degradation comes from a recent report[123] that labelled allopregnenolone was isolated from ripe tomato fruits into which tomatine-[4-$^{14}$C] had earlier been injected Since tomatidine can be degraded *in vitro* to yield allopregnenolone the above findings raise the possibility that *in vivo* degradation may occur in a similar manner Differences have been recorded in the biological activity and IR spectrum of authentic tomatine and tomatine from ripe tomato fruits[124] As well as having a lower haemolytic index, the latter gave a spectrum indicative of changes in the carbohydrate moiety The enzyme(s) responsible for breakdown of tomatine in ripening fruits has (have) not been isolated but a tomatine-hydrolysing enzyme has been detected in tomato leaves[125] This enzyme called tomatinase was found to exhibit a certain degree of substrate specificity and would not hydrolyse the steroidal glycoalkaloids solanine (**21**) or demissine (the β-lycotetraoside of **11**)




## BIOLOGICAL ACTIVITY

### *Microorganisms*

Research into the biological activity of tomatine was initiated by the finding that a crude extract of tomatine inhibited *in vitro* growth of *F. oxysporum* f. *lycopersici*[3] These same authors later showed[4] that impure tomatine also inhibited the growth of a number of bacteria and plant- and animal-pathogenic fungi, but not of *Escherichia coli* or *Penicillium notatum* However Fontaine *et al.*[6] found that low concentrations of partially-purified tomatine did inhibit *E. coli* and *P. notatum* while inexplicably higher concentrations did not The partially-purified alkaloid also proved highly effective *in vitro* against certain human dermatophytic fungi and various other fungi and yeast-like forms which cause internal disease in humans slightly effective against gram-positive and gram-negative bacteria certain fungi and plant-pathogenic *Actinomyces*, but without effect on human-pathogenic *Actinomyces* and fungi causing chromoblastomycoses Partially-purified tomatine

[121] SANDER H and ANGERMANN B (1961) *Tagungsber. Deut. Akad. Landwirtschaftswiss. Berlin* (27) 163
[122] SANDER H (1963) *Planta Med.* **11**, 23
[123] HEFTMANN E and SCHWIMMER S (1972) *Phytochemistry* **11**, 2783
[124] KAJDEROWICZ-JAROSINSKA D and CZUCHAJOWSKI L (1968) *Herba Pol.* **14**, 171
[125] PROKOSHEV S M PETROCHENKO E I and PASHSHNICHENKO V A (1956) *Dokl. Akad. Nauk SSSR* **106**, 313

was also reported to possess greater anti-bacterial activity than the purified alkaloid, with solutions of the latter up to 1 mg $cm^{-3}$ having almost no effect on *E coli*[7] On the other hand, *Candida albicans* was found to be susceptible to pure tomatine but not to the impure form,[126] the lack of effect of the latter apparently being due to antagonism by rutin and quercitin which were also present in the extract More recent investigations of the effect of tomatine on *E coli* indicated that the alkaloid is also capable of inhibiting oxygen metabolism in this organism[127] Inhibition was greater with lactic acid as a carbon source than with glucose, suggesting that the alkaloid might be inhibiting malic dehydrogenase Truhaut *et al*[128] found gram-positive bacteria to be more sensitive to tomatine than gram-negative bacteria and Sackmann *et al*[129] have reported the successful use of tomatine for isolation of *Brucella* spp and pleuropneumonia-like organisms Tomatine completely inhibits the growth of oxytrichids (protozoans) at a concentration of 45 $mg\%$, with lower concentrations giving corresponding reductions in growth[130] A number of workers[131–133] have drawn attention to the particular sensitivity of *Trichophyton mentagrophytes* (a dermatomycete) to tomatine and, in fact, the alkaloid is generally more effective against certain fungi associated with human disease (and particularly the Dermatomycetes) than against *F oxysporum* f *lycopersici*

However, compared with other antibiotics (e g penicillin and streptomycin), *in vitro* inhibition by tomatine tends to be weak and non-specific and to vary according to the strain of microorganism used and the nature, both physical and chemical, of the culture media[129,134–137] Furthermore, the efficacy of tomatine *in vivo* appears to be even more limited Although capable of inhibiting *in vitro* growth of the animal-pathogenic fungi, *Blastomyces dermatitidis* and *Histoplasma capsulatum*, it is ineffective in the treatment of diseases caused by these organisms (viz systematic blastomycosis and systemic histoplasmosis)[138] It is also reported to be ineffective against *Endamoeba histolytica* in guinea pigs and common food-spoilage microorganisms[138]

*Plants*

Little work has been done on the effect of tomatine on plants Vendrig[139] claimed that tomatine shows auxin-like activity in the *Avena* coleoptile test but this has been disputed by Roddick[140] who was unable to find evidence of tomatine-enhanced elongation in several standard auxin bioassays, the only effect on elongation being an inhibitory one at higher concentrations of the alkaloid (*ca* $10^{-3}$ M) Of interest however, is the finding that $10^{-4}$ M tomatine alone had no effect on elongation of *Avena* coleoptile segments but

[126] Ma, R M and Fontaine, T D (1948) *Arch Biochem* **16,** 399
[127] Šicho V and Mrhova O (1961) *Tagungsber Deut Akad Landwirtschaftwiss Berlin* (27), 291
[128] Truhaut R, Schuster G and Tarrade, A M (1967) *Ann Pharm Franc* **25,** 711
[129] Sackmann, W, Kern, H and Wiesmann, E (1959) *Schweiz Z Allgem Pathol Bakteriol* **22,** 557
[130] Petrov K (1963) *Zentr Bakteriol Parasitenk, Abt II* **117,** 70
[131] Tschesche, R and Wulff, G (1965) *Z Naturforsch* **20b,** 543
[132] Chanussot P (1957) *Anales Asoc Quim Arg* **45,** 113
[133] Bustinza, F (1947) *Farm Nueva (Madrid)* **12,** 15
[134] Arneson, P A and Durbin, R D (1968) *Phytopathology* **58,** 536
[135] McKee R K (1961) *Tagungsber Deut Akad Landwirtschaftswiss Berlin No* 27 277
[136] Wolters B (1966) *Planta Med* **14,** 392
[137] Wolters B (1968) *Planta* **79,** 77
[138] Fontaine, T D, Schaffer, P S, Doukas, H M, Scott, W E, Ma, R M, Turkot, V A, DeEds, F, Wilson R H and Doolittle, S P (1955) *U S Dept Agr* **ARS 73-8,** 1
[139] Vendrig, J C (1964) *Nature* **203,** 1301
[140] Roddick, J G (1972) *Planta* **102,** 134

caused marked inhibition of IAA-enhanced elongation The effect of tomatine on the growth of cucumber seedlings has been investigated by Rezk and Ferenczy [141] Germination was not greatly affected but at higher alkaloid concentrations ($10^{-5}$ and $10^{-4}$ M) root growth was strongly inhibited and characteristic deformations of the root were apparent Inhibition was slightly more pronounced at pH 7 than at pH 5 Seedlings grown in the presence of tomatine also showed reduced hypocotyl elongation but this was thought to be an indirect effect of the alkaloid due to impaired root development

*Insects*

Infiltration of foliage leaves with tomatine has been used by a number of workers to investigate the effect of the alkaloid on feeding habits and mortality rates of insect predators and their larvae Infiltration of tomatine into leaves of the potato plant (*Solanum tuberosum*) at a concentration of 2 mM kg $^{-1}$ leaf was sufficient to cause a 50% reduction in the lamina devoured by larvae of the Colorado beetle (*Leptinotarsa decemlineata*) while 3 mM kg $^{-1}$ leaf resulted in 100% larval mortality [142] Wild type' potato beetles were repelled by 0 02 -0 8% tomatine in potato leaves although the repellent action was more marked against DDT-resistant beetles [143] Kuhn *et al* [144] also reported that potato leaves containing 0 5% tomatine were not devoured by potato beetle larvae However, tomatine appears to exert no repelling action against two other potato beetles viz *Epilachna spar sa* and *E. niponica* [145] Imbibition by nymphs of the potato leafhopper (*Empoasca fabae*) can be completely restricted by $10^{-2}$ M tomatine with increasing concentrations of the alkaloid causing an increase in nymphal mortality but tomatidine has no effect on either imbibition or mortality [146] Tomatine has also been shown to interfere with the growth and development of nymphs of the two-striped grasshopper (*Melanoplus bivittatus*)[147] and larvae of the mosquito (*Aedes aegypti*) [148] In spite of the repellent action and toxicity of tomatine solutions to a variety of insect species tomatine dust has proved generally ineffective as an insecticide in the field [138]

*Mammals*

In preliminary toxicity tests, guinea pigs survived parenteral administration of up to 10 mg of partially-purified tomatine although in most cases such administrations elicited undesirable pathological or physiological responses in the test animals [6] Studies with mice revealed the $LD_{50}$ values for intraperitoneal, oral and subcutaneous administration to be 25 500 and 1000 mg kg $^{-1}$ body weight respectively [129]

A comprehensive study of the pharmacological and toxicological properties of tomatine has been made by Wilson *et al* [149] Diets containing up to 0 4% tomatine fed to rats daily for 200 days caused no abnormalities, but a single oral dose of 1 g kg $^{-1}$ body weight resulted in death within 24 hr Doses of 250 mg kg $^{-1}$ body weight given daily for 5 days did not produce any abnormalities Subcutaneous injection of tomatine (up to 40 mg kg $^{-1}$

[141] REZK M R and FERENCZY L (1969) *Acta Biol Szeged* **15** 71
[142] KUHN R and LOW I (1961) *Nachrbl Dtsch Akad Landwirtschaftswiss Berlin* (27) 7
[143] STURCKOW B and LOW I (1961) *Entomol Exp Appl* **4**, 133
[144] KUHN R LOW I and GAUHE A (1950) *Ber* **83** 448
[145] BUHR H (1961) *Nachrbl Dtsch Akad Landwirtschaftswiss Berlin* (27) 309
[146] DAHLMAN D L and HIBBS E T (1967) *Ann Entomol Soc Am* **60**, 732
[147] HARLEY K L S and THORSTEINSON A J (1967) *Can J Zool* **45**, 305
[148] HARLEY K L S (1967) *Can J Zool* **45**, 1297
[149] WILSON R H POLLY G W and DEEDS F (1961) *Toxicol Appl Pharmacol* **3**, 39

body weight) resulted in the formation of abscesses whose sizes were approximately proportional to the amount of tomatine injected Larger abscesses tended to ulcerate

The $LD_{50}$ in mice, to which tomatine was administered intravenously, was 18 mg $kg^{-1}$ body weight, with lethal doses causing death within 0 5–2 min The most common responses to intravenous tomatine were a large decrease in blood pressure and fluctuations in respiratory rate Where the dose of tomatine was lethal, death was thought to be due to this drop in blood pressure, but with sub-lethal doses, the initial drop was followed by an equally rapid recovery Repeated intravenous administrations of tomatine occasionally caused haemolysis and the subsequent appearance of haemoglobin in urine and lung exudate The haemolytic properties of the alkaloid have been confirmed by *in vitro* studies with whole blood

Application of a 5% tomatine ointment to the eye of rabbits caused conjunctivitis, but this disappeared a few days after discontinuing the treatment Application of the same ointment to the skin did not cause irritation

When tomatine is fed to rats as 1% of their diet, there is a subsequent decrease in the uptake of dietary cholesterol by the liver and an increase in the rate of hepatic and intestinal cholesterol synthesis [150] The rate of sterol excretion also decreases, but secretion of bile acids is unaffected

Human serum cholinesterase is inhibited by tomatine,[151,152] and horse serum cholinesterase, but not erythrocyte cholinesterase, by tomatine and tomatidine [153] It has been suggested[153] that both the steroid nucleus and the amine function are required for inhibition (the latter possibly constituting the binding site with the enzyme) and also that the 3-hydroxyl group may play an important role Nevertheless, Wilson *et al* [149] do not believe that the cholinesterase-inhibiting action of tomatine accounts for its observed pharmacological activity Tomatine is also reported to show cytostatic activity in the Miyamura test [131]

Following the discovery that extracts of crown-gall-infected tomato plants exhibited anti-histaminic properties *in vitro*,[89] it was shown[89,90] that guinea pigs could be protected from the lethal effects of a histamine aerosol by intraperitoneal injection of such extracts Analyses of the extracts suggested the active principle to be tomatine,[89,90] and oral, subcutaneous and intramuscular administration of tomatine isolated from crown-gall-infected tomato plants proved to be effective in reducing inflammation induced by a variety of methods [154] However, more recently, a compound named gomatine has been detected in extracts of crown-gall-infected tomato plants[155] and found to be a more potent antihistamine than tomatine [156] The latter authors have postulated that gomatine, and not tomatine, is mainly responsible for the antihistaminic properties of extracts of crown-gall-infected plants However, it is questionable whether the use of crown-gall-infected plants is significant in relation to these findings

Tomatine also appears to be toxic to larger domesticated mammals Forsyth[157] infers that the acute illness and death he observed in pigs which had eaten green parts of tomato plants was a result of tomatine poisoning

[150] CAYEN, M N (1971) *J Lip Res* **12**, 482
[151] POKROVSKIĬ, A A (1956) *Biokhimiya* **21**, 683
[152] ORGEL W H (1963) *Lloydia* **11**, 36
[153] FAUCHER A and MONNET R (1967) *Compt Rend* **246** 2247
[154] FILDERMAN R B and KOVACS B A (1969) *Br J Pharmacol* **37**, 748
[155] WAKKARY J A GOODFRIEND L and KOVACS B A (1970) *Arch Intern Pharmacodyn* **183**, 289
[156] WAKKARY J A GOODFRIEND L and KOVACS B A (1970) *Arch Intern Pharmacodyn* **183**, 303
[157] FORSYTH A A (1968) *British Poisonous Plants* HMSO, London

## ROLE IN PLANT DISEASE AND PREDATION

Although earlier work[1,2] had indicated that the degree of inhibition of *F. oxysporum* f. *lycopersici* in culture by expressed juice from tomato plants was proportional to the wilt resistance of the variety of tomato used, Irving[158] was unable to establish a relationship between the level of tomatine in different varieties and the degree of resistance to *Fusarium* wilt. However he did note that whereas tomatine levels were maintained in infected resistant varieties, levels in susceptible varieties declined as the infection progressed, suggesting that wilt resistance might be related not so much to the absolute amount of tomatine, but more to the rate at which it could be elaborated in response to infection. Later Kern[104] confirmed the absence of a relationship between absolute tomatine level and wilt resistance and further proposed that the alkaloid was not sufficiently inhibitory to *F. oxysporum* f. *lycopersici* or present in high enough concentrations in roots or stems to play a major role in resistance to this fungus. In the opinion of McKee[135] there is (up till 1961) no convincing evidence that steroidal glycoalkaloids confer disease resistance on plants which elaborate them, even though such alkaloids may be able to inhibit *in vitro* growth of certain pathogens. Subsequent work involving wilt-inducing fungi has, on the whole, led to similar conclusions. For example, in the most recent study of this subject, Langcake *et al.*[75] found that tomatine was quite inhibitory to hyphal extension of *F. oxysporum* f. *lycopersici in vitro* but, following infection of the plant, the level of tomatine in stems and roots increased in susceptible cultivars as well as in resistant cultivars. They therefore "*consider it unlikely that tomatine is involved in resistance of tomato to* F. oxysporum *f. lycopersici*".

However, Arneson and Durbin[67] have suggested that, since tomatine levels are higher in leaf tissues than in stems or roots, the alkaloid may be more important in resistance to leaf-infecting fungi. Assuming uniform distribution throughout the leaf and within the cell, the concentration of tomatine has been estimated at $10^{-3}$ M[159] but it has also been pointed out[134] that localization within the cell or in certain leaf cells could result in sufficiently high concentrations of the alkaloid to inhibit fungal growth. *Septoria lycopersici*, the fungus which causes leaf spot of tomato, has been shown capable of detoxifying tomatine both *in vitro* and *in vivo*, by enzymatically hydrolysing one glucose molecule from the glycoside to yield $\beta_2$-tomatine[67]. The enzyme has been purified and characterized as a constitutive extracellular glucosidase (β-D-glucoside glucohydrolase, E.C. 3.2.1.21) which breaks the $\beta 1 \rightarrow 2$ linkage between the two glucose molecules of the tetrasaccharide[67,160]. Attention has also been drawn to its high degree of substrate specificity[160]. Investigations into the effect of tomatine on other tomato parasites, on fungi pathogenic to other plants and on common saprophytic fungi have shown that tomato pathogens are generally less sensitive to tomatine than are non-pathogens of this plant[134]. However, it is not known if lower sensitivity to the alkaloid is due in all cases to detoxification of tomatine by enzymatic hydrolysis or to other mechanisms.

The possibility that tomatine may be important in the resistance of tomato to infection by yet other pathogens has been raised by the findings of Mohanakumaran *et al.*[161]. These authors found that tomatine levels are higher in roots of *L. pimpinellifolium* cultivars resistant to *Pseudomonas solanacearum* (which causes bacterial wilt) than in susceptible culti-

[158] IRVING, G. W. (1947) *J. Wash. Acad. Sci.* **37**, 293
[159] HEITMANN, L. (1967) *Lloydia* **30**, 209
[160] DURBIN, R. D. and UCHYTIL, T. F. (1969) *Biochim. Biophys. Acta* **191**, 176
[161] MOHANAKUMARAN, N., GILBERT, J. C. and BUDDENHAGEN, I. W. (1969) *Phytopathology* **59**, 14

vars Furthermore, alkaloid levels in roots of resistant cultivars increase following infection by this bacterium whereas those in roots of susceptible cultivars remain constant or decrease During ripening of tomato fruits, there is a decrease in their resistance to infection by *Colletotrichum phomoides*[162] but whether or not this is related to the decline in tomatine content of the fruits which also occurs during ripening is not yet known

A number of workers (e g Schreiber [163] Fraenkel[164 165]) believe that steroidal alkaloids may confer protection against insect predators Although Colorado beetles and their larvae die of alkaloid poisoning soon after devouring leaves of *Solanum auriculatum*,[163] it has also been proposed[166–168] that under natural conditions steroidal alkaloids, including tomatine, act as repellents rather than as toxins It may be significant, especially in relation to the findings of Arneson and Durbin[134] mentioned above, that the potato leafhopper[146] and the Colorado beetle[164] are less sensitive to the alkaloids of the potato plant (and especially to solanine) than to tomatine In view of the well-established insect-attractant properties of various isoprenoids, it has been questioned[164] whether it is any less likely that certain secondary products act as insect repellents

## MODE OF TOXIC ACTION

The toxicity of tomatine to a wide range of organisms is well documented, but the mechanism of toxicity is not yet fully understood Fontaine *et al* [7] attributed the antibiotic properties of tomatine to the aglycone moiety and, in fact, tomatidine appears to be more toxic than the glycoside to certain fungi[169] and to mice, when administered intravenously [149] On the other hand, tomatidine, unlike tomatine, does not affect imbibition by, or survival of, the potato leafhopper[146] and was less inhibitory than the glycoside to three test fungi (viz *Colletotrichum orbiculare*, *Septoria lincola* and *Helminthosporium turcicum*) used by Arneson and Durbin [170] More work is still required to elucidate the reasons for these differences

Because it consists of a hydrophobic steroid moiety linked to a hydrophilic sugar moiety, tomatine possesses surfactant properties similar to those of saponins McKee[171] has attributed the toxicity of steroidal glycoalkaloids (including tomatine) to fungal spores to such properties The fact that the less toxic hydrolysis products of tomatine (viz $\beta_1$-tomatine, $\beta_2$-tomatine and tomatidine) show less surface activity than the glycoside, is in keeping with such a mechanism, but at the same time, there are indications that differences in surface activity cannot fully account for differences in toxicity [170]

Following the discovery that tomatine can complex with 3$\beta$-hydroxy steroids,[50] it was proposed[163] that this might be the molecular basis of its toxicity (and possibly also that of other steroidal glycoalkaloids) Thus the mechanism of toxicity of tomatine has been compared[136] to that of polyene antibiotics which act by complexing with membrane sterols and altering or destroying membrane permeability Species of *Pythium* and *Phy-*

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*tophthora* which are insensitive to polyene antibiotics[172,173] presumably because their membranes lack sterols are also unaffected by tomatine.[174] Tomatidine, $\beta_1$-tomatine, $\beta_2$-tomatine and the protonated form of tomatine which exists at low pH do not complex with sterols[170] which may explain their reduced toxicities. Nevertheless these compounds are slightly toxic in varying degrees to different organisms possibly due to surfactant and/or other properties. It appears therefore that the unprotonated form of $\alpha$-tomatine may have two modes of toxic action (viz. sterol binding and surface activity) both of which disrupt the integrity of cell membranes. However the relative contribution of these two modes of toxicity to the lytic action of tomatine on for example fungal spores[171] mammalian erythrocytes[149] and pigmented plant tissues[175] is not yet known.

## APPLICATIONS

As a result of its fungicidal properties it was suggested that tomatine might be of value as a therapeutic agent in human disease.[4] However subsequent toxicity tests have ruled out parenteral administration and restricted its possible clinical use to topical or oral application.[149] A 0.5% hydrophilic tomatine ointment has been marketed by an Argentinian company and recommended for topical use in the treatment of mycotic dermatosis.[176] The report states the ointment to be especially effective in the treatment of Eczema, Tinea trichophytina, Tinea circinata, Tinea favosa, Favus of body and nails, Tinea microscopic, Intertrigo and onyx blastomycetes, Pityriasis versicolor and Erythrasma, without giving rise to odour, local irritations, secondary toxicity or cutaneous sensitivity.[138] The alkaloid can also be used at the same concentration as a powder or lotion. However no details of its clinical efficacy appear to be available.[149]

The authors[50] who first reported the ability of tomatine to complex with 3$\beta$-hydroxy steroids suggested that the alkaloid might be used for the precipitation and preparation of certain sterols. It has since been shown[177-181] that tomatine can be successfully employed for isolation and assay of cholesterol and there are claims[177,180,181] that it is even more specific than digitonin for the preparation of this sterol.

The fact that tomatidine can be chemically degraded to allopregnenolone[9,12,13,34-40] makes it a possible starting material for the synthesis of steroidal hormones and in fact progesterone[182,183] and epiandrosterone[184] have been partially synthesized from degradation products of tomatidine or its derivatives. Due to its lack of a $\Delta^5$ double bond tomatidine is not as convenient a starting material for steroidal hormone synthesis as is diosgenin (**22**) or solasodine (**12**) but with the increasing demand for new sources of synthetic steroid precursors", it may eventually prove a useful alternative. Since tomatine can

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be broken down to allopregnenolone *in vivo*, it has been suggested[123] that allopregnenolone itself might be extracted from waste tomato vines which have been suitably incubated to effect this degradation


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