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ANTINEOPLASTIC AGENTS FROM PLANTS

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INTRODUCTION

Since at least 1500 BC plants and plant extracts have been recognized as having anticancer activities (1). Surveys by Hartwell (2, 3) listed at least 3000 species so used. However the rational, organized study of plants as sources of potential antineoplastic agents probably commenced with the pioneering studies of Hartwell et al (4–8) during the period 1947–1953 in which for the first time pure plant constituents were isolated, characterized, and associated with the antitumor activity of the crude plant extract.[1]

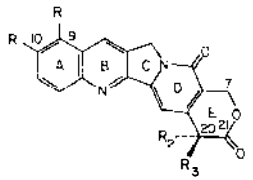
This review presents a critical appraisal of the current status of plant antineoplastic agents, with particular emphasis on chemical structure and the significant features (where known) that are required for antitumor activity. This review deals only with plant antineoplastic agents of high activity against mouse leukemia and mouse and rat solid tumors as defined by the National Cancer Institute (9). All of the plant antitumor agents covered have excellent antitumor activity in one or more rodent tumor or leukemia systems combined with reasonable therapeutic indices so that the agents are either in clinical testing or scheduled for such studies. As a consequence, many substances with marginal activity or with high toxicity are not included in this review. Material covered in earlier reviews (1, 10) is not discussed unless additional information has become available since the time of the previous review.

[1]We wish to dedicate this review in honor of Dr. Jonathan L. Hartwell, who retired from the National Cancer Institute in January 1975, after more than 30 years of distinguished service during which he not only pioneered in the rational investigative studies of plant antitumor agents but also provided continuous stimulation and support to many other workers in the field.

ALKALOIDS

Camptothecin and Related Alkaloids

Camptothecin I was isolated by Wall et al from the wood and bark of *Camptotheca acuminata,* a tree which is a native of China (11). The alkaloid is biogenetically related to the well-known indole alkaloid group. The compound is highly active in the L-1210 and P-388 mice leukemia systems as well as against a number of experimental rodent tumors, including Walker 256 carcinosarcoma, Lewis Lung carcinoma, and melanotic melanoma B-16. Wani & Wall (12) isolated the 10-hydroxy and 10-methoxy analogues II and III as minor components of *C. acuminata.* The 9-methoxy analogue IV has also been isolated from *Mappia foetida* (13).



I, $R = R_1 = H$; $R_2 = C_2H_5$; $R_3 = OH$

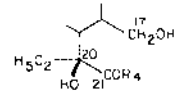
II, $R = OH$; $R_1 = H$; $R_2 = C_2H_5$; $R_3 = OH$

III, $R = OCH_3$; $R_1 = H$; $R_2 = C_2H_5$; $R_3 = OH$

IV, $R = H$, $R_1 = OCH_3$, $R_2 = C_2H_5$, $R_3 = OH$

V, $R = R_1 = H$; $R_2 = C_2H_5$; $R_3 = Cl$

VI, $R = R_1 = H$; $R_2 = C_2H_5$; $R_3 = H$



VII, RINGS ABCD AS IN I, $R_4 = ONa$

VIII, RING ABCD AS IN I, $R_4 = NHCH_3$

Wall (14) has shown that the 20-hydroxyl and the lactone moiety found in Ring E of camptothecin are an absolute requirement for antileukemia activity. The 20-chloro and 20-desoxy analogues V and VI were devoid of activity[2] as was the lactol obtained by reduction of the 21-carbonyl. The 20-ethyl substituent is not an absolute requirement for activity. Thus, Sugasawa et al (15) have shown that the ethyl group can be substituted by allyl, propargyl, and several other substituents, with allyl being the most active. Sugasawa et al confirmed the requirement of the α-hydroxy lactone moiety for activity (15). Compounds, which can regenerate ring E on acidification, such as the sodium salt VII, and the methyl amide VIII, are also active although their activity is less than the parent compound I. Oxygenated ring A analogues II, III, and IV are highly active in life prolongation when tested against mice leukemia systems. A large number of synthetic analogues have been prepared, including

[2]Desoxycamptothecin VI was reported by Hartwell & Abbott (1) to be active against L-1210. The data quoted by these workers were obtained from a compound prepared by Wall et al (11) which Wall and Wani (unpublished data) found to have 5% camptothecin as an impurity. A later sample of VI prepared by Wall and Wani that was extremely pure was found to be inactive in L-1210 leukemia. Unfortunately the original observation (1) has been widely quoted and as the individuals responsible for *both* preparations of VI, we wish to clarify the situation.

bicyclic (ring DE) analogues (16, 17) and tricyclic (ring CDE) analogues (17) which contain the vital hydroxy lactone moiety. Schultz (18) and Shamma & St. Georgiev (19) have extensively reviewed the various synthetic methods and compounds. The bicyclic (16) and tricyclic analogues (17) are inactive as antitumor agents, indicating that factors other than the α-hydroxy lactone moiety are involved. In this connection the extensive studies of Horwitz and her collaborators regarding the effect of I and its analogues on nucleic acid synthesis are very important [cf (20) for a review and complete literature citations]. It was found that camptothecin I was a potent inhibitor of nucleic acid synthesis in HeLa cells and in L-1210 cells. Compound I degrades cellular DNA to lower molecular weight species and also inhibits RNA syntheses. Unlike the rather specific structural requirements for antitumor activity, inhibition of RNA synthesis and DNA degradation is brought about by a number of camptothecin analogues. The requirement for activity seems to be rings ABCD as in I, but the α-hydroxy lactone moiety is not an absolute requirement (20).

The antileukemic properties of camptothecin may be due to several features of the structure of this compound. Thus rings ABCD comprise a flat planar structure which may produce an intercalation effect on DNA similar to that found in the case of aromatic planar bacterial inhibitors such as acridine and proflavine [cf (21) pp. 269–75 for an excellent discussion of this subject]. In addition, however, camptothecin possesses an *alkylating moiety.* The lactone ring of camptothecin is facilely attacked by bases under mild conditions, possibly because of the activating effect of the α-hydroxyl group (1, 14). The potent combination of an alkylating moiety combined with a nucleus capable of intercalation with the DNA helix may well account for the total activity of I. Unfortunately, because of its water insolubility, camptothecin has been tested clinically only as the water-soluble sodium salt VII, which recent studies (M. E. Wall and M. C. Wani, unpublished results) have shown to be much less active than I, II, or III.

Dimeric Benzylisoquinoline and Related Alkaloids

The antitumor activity of this group of alkaloids has been established largely as a result of the researches of Kupchan and his co-workers. Thalicarpine IX is a novel dimeric aporphine-benzylisoquinoline alkaloid isolated from *Thalictrum dasycarpum* Fisch. and Lall. (22), and from other Thalictrum species; its structure was elucidated by Tomita et al (23). Tetrandrine X is a dimeric bis(benzylisoquinoline) alkaloid isolated from *Cyclea peltata* Diels (24). Both of these alkaloids showed significant inhibitory activity against the Walker intramuscular carcinosarcoma-256 (1), and after several years of preclinical toxicological studies, these compounds are on clinical trials (25).

Recent studies have defined some of the structural requirements for tumor-inhibitory activity among benzylisoquinoline alkaloids and related compounds (26). The monomeric benzylisoquinolines and aporphines do not show any tumor-inhibitory activity against the W-256 tumor system. Thus, XI and the aporphine alkaloids

IX

X

XI

XII, R = CH_3
XIII, R = H

XIV, $R^1=R^3=CH_3$; $R^2=H$
XV, $R^1=R^2=CH_3$; $R^3=H$
XVI, $R^1,R^2=-CH_2-$; $R^3=H$

XVII

XVIII

XIX

XXa, R = CH_3; R' = H
XXb, R = R' = H
XXc, R = R' = CH_3

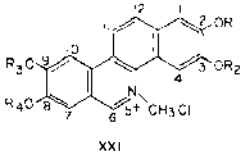
glaucine XII, boldine XIII, corydine XIV, isocorydine XV, and bulbocapnine XVI were all inactive. Since both IX and X exhibit equally good activity, it appears that the two components of dimeric alkaloids could be either benzylisoquinoline or aporphine. Thalidaisine XVII is another bisbenzylisoquinoline alkaloid isolated from *Thalictrum dasycarpum* (27). The comparable activities of X and XVII against W-256 suggest that the size of the macrocyclic ring in these alkaloids is not important. In fact, according to Kupchan et al (26), the macrocyclic ring may not be required because *dl*-O-methyldauricine XXa is about as active as X and XVII. An alkyl substituent at the nitrogen atoms is not necessary because XXb and its precursor XVIII are both active. The activity of XVIII also indicates that stereospecificity is not required.

In view of the known sensitivity of the W-256 system toward alkylating agents, it was considered likely that the bis(benzylisoquinoline) alkaloids may exert their tumor-inhibitory activity by bisalkylation of biological nucleophiles (26). This would involve in vivo dehydrogenation to the electrophilic bis(dihydroisoquinolinium) system present in XIX. However, XIX itself was found to be inactive, and XXC which cannot undergo ready transformation to a compound like XIX, was found to be active.

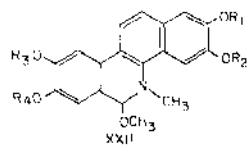
Benzophenanthridine Alkaloids

Nitidine chloride XXIa was first isolated from *Zanthoxylum nitidium* (28) and more recently from *Fagara macrophylla* (29). Both XXIa and a 6-methoxy analogue XXIIa isolated as an artifact during the extraction process (29) are highly active against P-388 leukemia; the latter is also active in the L-1210 leukemia system. A related alkaloid fagaronine XXIb (30, 31) isolated from *F. zanthoxyloides* also shows excellent antitumor activity. On the other hand although sanguinarine XXIIIa and its salt XXIVa are cytotoxic, they are devoid of activity against in vivo tumor systems (J. L. Hartwell, National Cancer Institute, private communication).

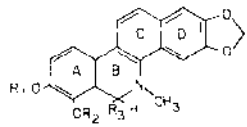
In an effort to gain an insight into structure-activity relationships, a number of benzophenanthridine derivatives related to nitidine chloride and sanguinarine have been synthesized and evaluated for antileukemic activity. The antileukemic activity of four analogues of nitidine and the corresponding 6-methoxy-5,6-dihydro derivatives has been reported (32). Allonitidine XXIc and the tetramethoxy analogue XXId are active against P-388 and L-1210 leukemia systems. Zee-Cheng & Cheng (32) report that the antileukemic activity of XXId is slightly greater than that of XXIa which, in turn, shows slightly higher activity than XXIc. The tetrahydroxy analogue XXIe and the tetraacetoxy analogue XXIf are both inactive. The activity of 6-methoxy-5,6-dihydro analogues XXIIa, XXIIc, and XXIId is slightly higher than that of the corresponding salts XXIa, XXIc, and XXId. There is a marked difference between the solubility of the salts and the corresponding 6-methoxy analogues in water. Whether this solubility difference is responsible for the somewhat greater antitumor activity of the latter group of compounds is not clear.



XXI



XXII



a, $R_1 + R_2 = CH_2$; $R_3 = R_4 = CH_3$

b, $R_1 = H$; $R_2 = R_3 = R_4 = CH_3$

c, $R_1 = R_2 = CH_3$, $R_3 + R_4 = CH_2$

d, $R_1 = R_2 = R_3 = R_4 = CH_3$

e, $R_1 = R_2 = R_3 = R_4 = H$

f, $R_1 = R_2 = R_3 = R_4 = COCH_3$

XXIII a, $R_1, R_2 = -CH_2-$; $R_3 = OH$

XXIII b, $R_1, R_2 = -CH_2-$; $R_3 = OCH_3$

XXIII c, $R_1, R_2 = -CH_2-$; $R_3 = OCH_2CH_3$

XXIII d, $R_1, R_2 = -CH_2-$; $R_3 = OCH_2CH_2CH_3$

XXIII e, $R_1, R_2 = -CH_2-$; $R_3 = OCH_2CH_2CH_2CH_3$

XXIII f, $R_1, R_2 = -CH_2-$; $R_3 = CH_2C(=O)CH_3$

XXIII g, $R_1, R_2 = -CH_2-$; $R_3 = H$

XXV a, $R_1 = R_2 = CH_3$; $R_3 = OH$

XXV b, $R_1 = R_2 = CH_3$; $R_3 = OCH_3$

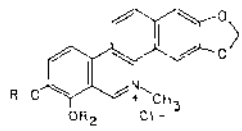
XXV c, $R_1 = R_2 = CH_3$; $R_3 = OCH_2CH_3$

XXV d, $R_1 = R_2 = CH_3$; $R_3 = OCH_2CH_2CH_3$

XXV e, $R_1 = R_2 = CH_3$; $R_3 = OCH_2CH_2CH_2CH_3$

XXV f, $R_1 = R_2 = CH_3$, $R_3 = CH_2C(=O)CH_3$

XXV g, $R_1 = R_2 = CH_3$; $R_3 = H$



XXIVa, $R_1, R_2 = CH_2$

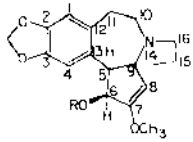
XXIVb, $R_1 = R_2 = CH_3$

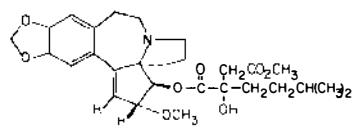
Stermitz and collaborators (33) have prepared and tested a series of sanguinarine analogues XXIIIb-XXIIIg, chelerythrine XXVa, and its analogues XXVb-XXVg and XXIVb for cytotoxicity and antileukemic activity (33). These compounds were weakly cytotoxic and failed to show any activity against P-388 and L-1210 leukemia systems. Nitidine chloride XXIa and chelerythrine chloride XXIVb differ from each other only in the position of one methoxyl group in ring A, the former having the 8,9-dimethoxy moiety, the latter the 7,8-dimethoxy moiety. Two possible explanations for the differences in the activity of the above compounds have been offered (33). A common structural feature among many antitumor alkaloids consists of a triangle formed by one nitrogen atom and two oxygen atoms with rather definite interatomic distances (34). The O–O bond distances in all the above derivatives of sanguinarine and chelerythrine fall within the accepted range. However, the N–O bond distances in all cases are shorter than the minimum range found in active compounds (33). The same distances in nitidine and its derivatives do fall within listed values for active antitumor agents. Be this as it may, the mechanism of antitumor activity in this alkaloid series may again be due to a combination of several factors; first a flat, planar structure amenable to intercalation (see section on camptothecin and related alkaloids) and second an alkylation site in the $-C-N^+$ region of ring B. In nitidine and analogous substances with 8,9-dimethoxyl substituents in ring A, it has been suggested that there would be no steric hindrance to alkylation of biological nucleophiles (33). In the inactive sanguinarine and chelerythrine series with substitution in the 7,8 position, the substituent at C-7 is in a position to block alkylation sterically at the C-6 position.

Cephalotaxus Alkaloids

This group of active antitumor alkaloids has a structural theme that occurs in a number of other active agents; there is an alcohol (usually a complex, multi-ring structure) that is esterified with a variety of aliphatic acids. Usually both components must be present for retention of activity.

The alkaloids of *Cephalotaxus harringtonia* var. *drupacea* (family Taxaceae) are a group of compounds with an unusual structure. The isolation and partial characterization of the parent alkaloid alcohol cephalotaxine XXVI was first reported by Paudler, Kerley & McKay (35). Subsequently the complete structure of this alkaloid was established by a combination of chemical and physical methods including X-ray crystallography (36–38). The alkaloid esters harringtonine XXVII, isoharringtonine XXVIII, homoharringtonine XXIX, and deoxyharringtonine XXX are minor components of the same plant.





XXVI, R = H

XXVII, R = $\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}H(OH)-CH_2CH_2C(CH_3)_2(OH)$

XXVIII, R = $\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}H(OH)-CH_2CH_2CH(CH_3)_2$

XXIX, R = $\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}H(OH)-CH_2CH_2CH_2C(CH_3)_2(OH)$

XXX, R = $\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}H(OH)-CH_2CH_2CH(CH_3)_2$

XXXI, R = $\overset{O}{\overset{\|}{C}}-CH_3$

XXXII, R = $\overset{O}{\overset{\|}{C}}-CH_2-\overset{CO_2CH_3}{\overset{|}{C}}(OH)-CH_2CH_2CH(CH_3)_2$

XXXIII, R = $\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\overset{|}{C}}(OH)-CH_2CH_3$

XXXIV, R = $\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}H(OH)-CH_2CH_2CH(CH_3)_2$

XXXV, R = $\overset{O}{\overset{\|}{C}}-CH(CH_2CO_2CH_3)-CH_2CH_2CH(CH_3)_2$

XXXVI

The harringtonines which are esters of cephalotaxine and substituted malic and tartaric acids exhibit significant activity against P-388 lymphocytic leukemia over a wide range of dosage (39, 40). Alkaloids XXVII, XXIX, and XXX show greatest activity at 1–2 mg/kg, XXVII being the most active. In the same system the alkaloid XXVIII has the greatest activity at 7.5 mg/kg. However, against L-1210 lymphocytic leukemia the alkaloids XXVII, XXVIII, and XXIX show only marginal

activity. The activity of XXX in this system is not reported. Cephalotaxine XXVI and acetylcephalotaxine XXXI (36) are both inactive in the P-388 leukemia system. The partially synthetic pseudo-deoxyharringtonine XXXII shows only marginal activity at 40 mg/kg. The three esters of cephalotaxine XXXIII, XXXIV, and XXXV (41) and the rearranged ester XXXVI (42) are also inactive, although XXXIII does show marginal activity at 135 mg/kg.

Based on the above results, structure-activity relationships in *Cephalotaxus* alkaloids have been reported (42). The inactivity of XXVI indicates that an ester function at C-6 is necessary for activity. Furthermore, the inactivity or substantially reduced activity of esters XXXI–XXXVI suggests a high degree of structural specificity in the acyl moiety. An α-hydroxyl group and a hindered tertiary carboxyl group in the acyl portion are absolutely required for activity (42) (compare, for example, XXX with XXXV). However, some variations in the acyl side chain are allowed. Insertion of an additional methylene group in the terminal portion of the side chain has little effect on the activity (compare, for example, XXVII with XXIX). Removal of the hydroxyl group from the penultimate carbon atom of the side chain causes a 50% reduction in activity (for example, compare XXVII with XXVIII). Shifting of the ester function from C-6 to C-8 results in an inactive molecule (for example, compare XXVII with XXXVI). It is attractive to propose that the ester function of the biologically active harringtonines not only is involved in processes such as transport or complex formation but also is a part of the electrophilic allylic ester system. The biological nucleophile probably attacks the double bond of the allylic system resulting in the migration of the double bond and elimination of the ester function as the carboxylate anion. Since the ester function is bulky, the latter process should be facilitated by the relief of steric strain. In this connection it is interesting to note that the reaction of cephalotaxine chloride (Cl instead of OH at C-6 in XXVI) with the silver salt of an appropriate half acid results in the formation of the rearranged ester XXXVI (42).

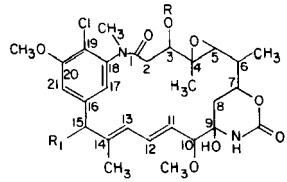
NONALKALOIDS

Groups included here are maytansine and related ansa macrolides, taxol and related diterpenoids, quassinoids and triptolides. Although the first two groups in the above series contain nitrogen, it is in a nonbasic form and hence the compounds are not classified as alkaloids. It will be seen that the maytansine, taxol, and the quassinoids all have ester groups which as in the case of the previously discussed harringtonine alkaloids is a requirement for any activity in some cases or in all cases for normal antitumor activity.

Maytansinoids

Maytansine XXXVII is a novel ansa macrolide isolated from several *Maytenus species* by Kupchan and co-workers (43, 44, 46). It has remarkably potent inhibitory activity against L-1210 and P-388 leukemias and several murine tumors at the level of micrograms per kilogram body weight over a wide dosage range (46). After preclinical toxicological studies, this compound recently has been selected for clinical trials. Maytansine is the first ansa macrolide containing carbinolamide, epoxide,

and aryl halide functions. The unusual biological activity of maytansine stimulated interest in the isolation of related maytansinoids.



XXXVII , R = CO.CHCH$_3$.NCH$_3$.CO CH$_3$, R_1 = H

XXXVIII , R = CO.CHCH$_3$.NCH$_3$.CO CH$_2$CH$_3$, R_1 = H

XXXIX , R = CO.CHCH$_3$.NCH$_3$.COCH(CH$_3$)$_2$, R_1 = H

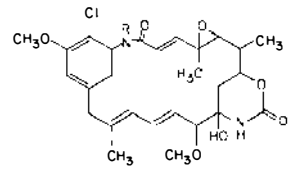
XL , R = CO.CHCH$_3$.NCH$_3$.COCH(CH$_3$)$_2$, R_1 = OH

XLI , R = CO.CHCH$_3$.NCH$_3$.COCH(CH$_3$)$_2$, R_1 = OAC

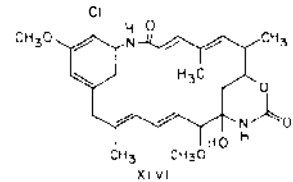
XLII , R = CO.CHCH$_3$.NCH$_3$.COCH$_2$CH(CH$_3$)$_2$, R_1 = H

XLIII , R = COCH$_3$, R_1 = H

XLIV , R = R_1 = H



XLV, R = CH$_3$
XLVI, R = H



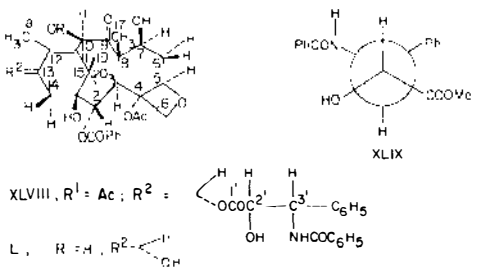
XLVI

Recent studies have resulted in the isolation of ten new maytansinoids. From the antitumor activities of these compounds several deductions can be made concerning structure-activity relationships. Thus, an ester function at C-3 is absolutely necessary for activity. For example maytansine XXXVII, maytanprine XXXVIII (44), maytanbutine XXXIX (44), colubrinol XL (47), colubrinol acetate XLI (47), maytanvaline XLII (45), and maytanacine XLIII (46) exhibit potent antileukemic activity. On the other hand, the parent alcohol maytansinol XLIV (46), maysine XLV (45), normaysine XLVI (45), and maysenine XLVII (45) which lack the ester function have no antileukemic activity. Kupchan and co-workers have recently shown (46) that an amino acid residue at C_3 is not an absolute requirement and that the simple acetic acid ester XLIII is highly potent. It has been suggested (45) that the ester function in maytansinoids may be involved in the formation of selective

molecular complexes with enzymes. The formation of molecular complex may be necessary for the selective alkylation of specific nucleophiles by the carbinol amide and epoxide functions. In support of this hypothesis, maytansine ethyl ether XXXVII (OC_2H_5 instead of OH at C-9) in which the carbinolamide is no longer available for alkylation is devoid of antileukemic activity. Thus we may conclude that the carbinolamide moiety is also absolutely required for activity. The role of the 4,5-epoxide and the 19-chloro substituent remains to be defined. Wani, Taylor & Wall (47) have shown that 15-hydroxy or 15-acetoxy substituents do not affect antineoplastic activity.

Taxane Diterpenoids

Taxol XLVIII, a complex ester isolated from several species of the genus *Taxus* (family Taxaceae) including *T. brevifolia, T. cuspidata,* and *T. baccata,* has been shown to be a taxane derivative containing a rare oxetan ring (48). Taxol shows confirmed activity against L-1210, P-388, and P-1534 leukemia systems, being highly active against the latter two systems. It is also a potent inhibitor of WM-256 carcinosarcoma and shows considerable cytotoxicity in 9KB assay ($ED_{50} = 5.5 \times 10^{-5}$ μg/ml). Taxol has been selected for advanced preclinical pharmacology by the National Cancer Institute. A mild base-catalyzed methanolysis of taxol gives the methyl ester XLIX and the tetraol L. The latter is also found in the plant (M. E. Wall, M. C. Wani, H. L. Taylor, unpublished results). The cytotoxicities of XLIX and L show that XLIX is inactive and the taxane L to be only one thousandth as active as taxol.

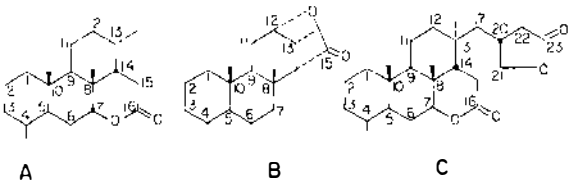


On the basis of limited data, it is interesting to speculate that the activity of taxol is also due to the allylic α-hydroxy ester function. The ester could form molecular complexes and also act as a leaving group developing a positive charge at C-13, thus forming an alkylating center of considerable specificity. Recently the isolation and characterization of a number of oxetan-containing taxane derivatives have been reported (49). Unfortunately, biological data, if any, on these compounds are not available. Therefore, a meaningful structure-activity relationship in this class of compounds is not possible.

Quassinoids

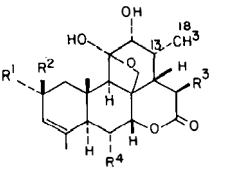
The bitter principles of the simaroubaceae family, which are degraded triterpenoids, are known as the quassinoids. During the last fifteen years, a number of new

constituents have been isolated from many genera of this family (50). Most of the quassinoids are fundamentally C_{20}-compounds with basic skeleton A. Some are C_{19}-compounds with basic skeleton B and only two are C_{25}-compounds with basic skeleton C. A number of bitter principles have been known to possess pharmacological activity and in particular antiamoebic activity (51). Recent studies have shown that certain members with basic skeleton A display antitumor activity.



A B C

The esters of glaucarubolone such as holacanthone LI (52), glaucarubinone LII (53), 2'-acetylglaucarubinone LIII (53), ailanthinone LIV (53), dehydroailanthinone LV (53), and undulatone LVI (M. E. Wall, M. C. Wani, and H. L. Taylor, unpublished information) are active against P-388 lymphocytic leukemia. However, glaucarubolone LVII, which lacks an ester function at C-15, and glaucarubin LVIII, which has an ester function at C-15 but lacks the conjugated carbonyl in ring A, show a much lower order of activity (M. E. Wall, M. C. Wani, and H. L. Taylor, unpublished information). Saturation of the 3,4 double bond in the quassinoids also resulted in a decrease in cytotoxicity (53).



LI , $R^1, R^2 = O$, $R^3 =$ OAc , $R^4 = H$

LII , $R^1, R^2 = O$, $R^3 = O_2CC(OH)(CH_3)-Et$, $R^4 = H$

LIII , $R^1, R^2 = O$, $R^3 = O_2CC(OAc)(CH_3)-Et$, $R^4 = H$

LIV , $R^1, R^2 = O$, $R^3 = O_2CCH(CH_3)-Et$, $R^4 = H$

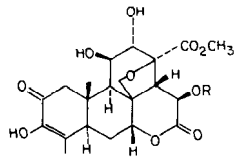
LV , $\Delta^{13,18}$, $R^1, R^2 = O$, $R^3 = OCCH(CH_3)-Et$, $R^4 = H$

LVI , $R^1, R^2 = O$, $R^3 =$ OAc, $R^4 = O_2CC(CH_3)=CH-CH_3$

LVII , $R^1, R^2 = O$, $R^3 =$ OH , $R^4 = H$

LVIII , $R^1 =$ OH , $R^2 = R^4 = H$, $R^3 = O_2CC(OH)(CH_3)-Et$

While the antileukemic activity of the esters of glaucarubolone is not affected significantly by the nature of the ester substituents, that of the esters of bruceolide seems to vary greatly with the ester function (54). Thus, bruceantin LIX and bruceantinol LX containing α,β-unsaturated ester moieties are potent antileukemic agents. Bruceantarin LXI with a benzoate ester is moderately active, whereas bruceine B LXII with a smaller acetate ester and bruceolide LXIII with no ester show only marginal activity. Bruceolide and its congeners differ from glaucarubolone and



LIX, R = COCH=C(Me)–CH(CH$_3$)$_2$

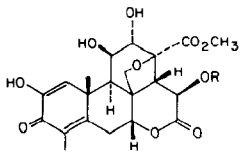
LX, R = COCH=C(Me)–C(OH)(CH$_3$)$_2$

LXI, R = COC$_6$H$_5$

LXII, R = Ac

LXIII, R = H

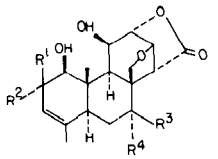
its congeners in two respects: (*a*) the hydroxyl group is present at C-3 instead of at C-1; (*b*) the hydroxymethyl group at C-8 is present as an ether bridge to C-13 instead of a hemiketal bridge to C-11. These structural changes seem to be compatible with biological activity. On the other hand, dehydrobruceantin LXIV and dehydrobruceantarin LXV show only marginal activity suggesting that this modification of ring A destroys activity.



LXIV, R = COCH=C(CH$_3$)–CH(CH$_3$)$_2$

LXV, R = COC$_6$H$_5$

The C_{19} quassinoids (50), samaderin C LXVI and cedronine LXVII, both of which lack an ester function, are devoid of activity against P-388 leukemia system (M. E. Wall, M. C. Wani, and H. L. Taylor, unpublished results).

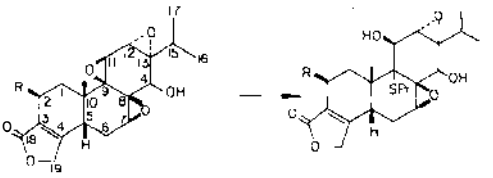


LXVI, R^1=H, R^2=OH, R^3, R^4=O
LXVII, R^1, R^2=O, R^3=H, R^4=OH

Studies regarding the isolation, characterization, and biological activity of quassinoids are continuing in our laboratory. The results obtained to date suggest that (*a*) an ester group adjacent to the lactone carbonyl and (*b*) a conjugated ketone seem absolutely required for antitumor activity in these compounds. As is the case with maytansinoids (cf section on maytansinoids), the ester moiety probably serves as a carrier group in processes such as transport or complex formation (54). The highly electrophilic conjugated ketone is probably involved in the alkylations of biological nucleophiles.

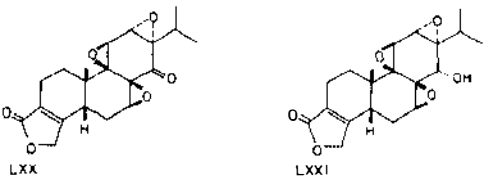
Triptolides

Triptolide LXVIII and tripdiolide LXIX are novel antileukemic diterpenoid triepoxides isolated from *Tripterygium wilfordii* (55). Both of these compounds exhibited very high activity against L-1210 at 0.1 mg/kg. Triptolide LXVIII is currently undergoing advanced pharmacological studies in preparation for clinical trials. The triptonide LXX, which has a carbonyl function at C-14, is inactive. The minor variants epitriptolide LXXI and the thiol adducts LXXII and LXXIII are also inactive (56).



LXVIII, R = H
LXIX, R = OH

LXXII, R=H
LXXIII, R=OH



LXX

LXXI

All the above triptolides contain electrophilic epoxide and α,β-unsaturated ketone moieties which have been shown to be important for the tumor-inhibiting activity of several classes of terpenoids (57, 58). However, the active triptolides LXVIII and LXIX are the only two that contain a characteristic hydrogen bonded 9,11-epoxy-14-β-hydroxy system. The presence of strong intramolecular hydrogen bonding in LXVIII and LXIX is indicated by NMR spectroscopy. It is proposed that intramolecular catalysis by the 14β-hydroxyl group may assist selective alkylation of biological macromolecules by the 9,11-epoxide (56). In support of this hypothesis, treatment of LXVIII and LXIX with propanethiol resulted in the formation of LXXII and LXXIII respectively by preferential nucleophilic attack at C-9. Similar treatment of the 14-epimeric derivative LXXI gave no reaction.

CONCLUSIONS

The preceding sections cover the structure-activity relationships of a relatively small number of highly active antineoplastic agents of plant origin. In most cases the structural features required for activity have been elucidated. In almost every case a definite alkylation center could be identified. A common theme involved aromatic planar structures which not only could act as intercalation agents but also contained an alkylation site. Another structural feature of frequent occurrence was an ester group (which did not have antineoplastic activity per se) but which was absolutely required for overall activity. Although thousands of plants have been studied, only a small number of the total plants available have been surveyed. Undoubtedly many new agents will be discovered in the future. The current active agents and new compounds as their structures are unraveled will also serve as templates for synthetic modifications which may lead to more active and/or less toxic compounds.

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