SYNTHESIS OF THE ANTITUMOR DIMERIC INDOLE ALKALOIDS FROM *CATHARANTHUS* SPECIES (VINBLASTINE GROUP)[1]

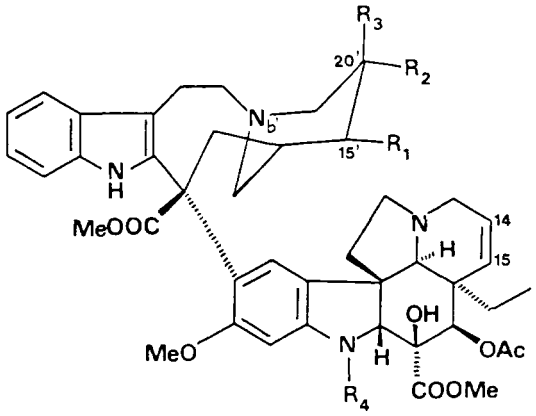
PIERRE POTIER

Institut de Chimie des Substances Naturelles du C. N. R. S., 91190—Gif-sur-Yvette, France

ABSTRACT.—The extensive use of a reaction previously discovered in our laboratory (i.e. the modified Polonovski reaction) has led us to achieve the synthesis of all the significantly bioactive alkaloids of the vinblastine group. The strategy used and the various syntheses will be described as well as the preparation of a completely new series of derivatives: the nor-vinblastine series.

The use of the Madagascan periwinkle (*Catharanthus roseus* G. Don, Apocynaceae, also known as *Vinca rosea* L.) as an hypoglycemic agent has provided the stimulus for the discovery of the antitumor activity of alkaloidal extracts from this plant. This pioneering research has been described at length (1) by several of the people responsible for this early work.

During the course of studies directed towards the search for this reported hypoglycemic activity, a strong granulocytopaenia developed in the treated animals. Further research led to the isolation of one of the bis-indole alkaloids partly responsible for this activity: vincaleucoblastine or vinblastine **1** (1, 2).



1: R_1 = H R_2 = Et R_3 = OH R_4 = Me : **VINBLASTINE**
2: R_1 – R_2 = O R_3 = Et R_4 = Me : **LEUROSINE**
3: R_1 = H R_2 = Et R_3 = OH R_4 = CHO : **VINCRISTINE**
4: R_1 = H R_2 = OH R_3 = Et R_4 = Me : **LEUROSIDINE**

Other molecules of the same type were discovered shortly after: leurosine **2** (3), leurocristine or vincristine **3** (2) and leurosidine or vinrosidine **4** (1).

The structural elucidation of these complex molecules is mainly due to the

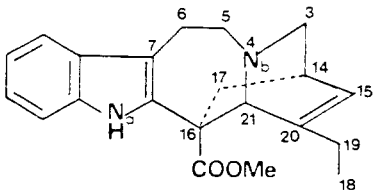
[1]This paper was presented as a plenary lecture at the Twentieth Annual Meeting of the American Society of Pharmacognosy at Purdue University, West Lafayette, Indiana, July 30-August 3, 1979.

efforts of Neuss, Svoboda, Gorman, Büchi and their coworkers. This beautiful piece of research has been reviewed (1, 4).

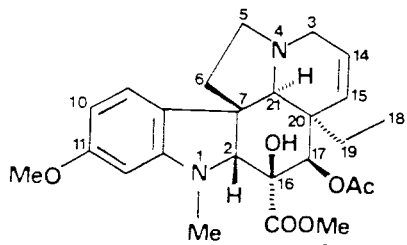
Vinblastine **1** and vincristine **3** have been in use for more than twenty years in the treatment of various leukemias, Hodgkin's disease and solid tumors.

The natural abundance of these products is rather low: some grams per ton of dried plant material. These compounds are consequently among the most expensive drugs on the pharmaceutical market. It is, therefore, in no way surprising that a considerable research effort has been devoted to the partial or total synthesis of these precious chemicals.

For this purpose, it was initially assumed that vinblastine-type compounds could well be derived from their two obvious precursors: catharanthine **5** and vindoline **6** (14), which are the major monomeric tertiary bases present in the aerial parts of the various *Catharanthus* species examined so far. Vinblastine-type compounds can indeed be considered as resulting from the nucleophilic attack of vindoline **6** on a convenient catharanthine derivative, for example, 16,21-secocatharanthine **9**.

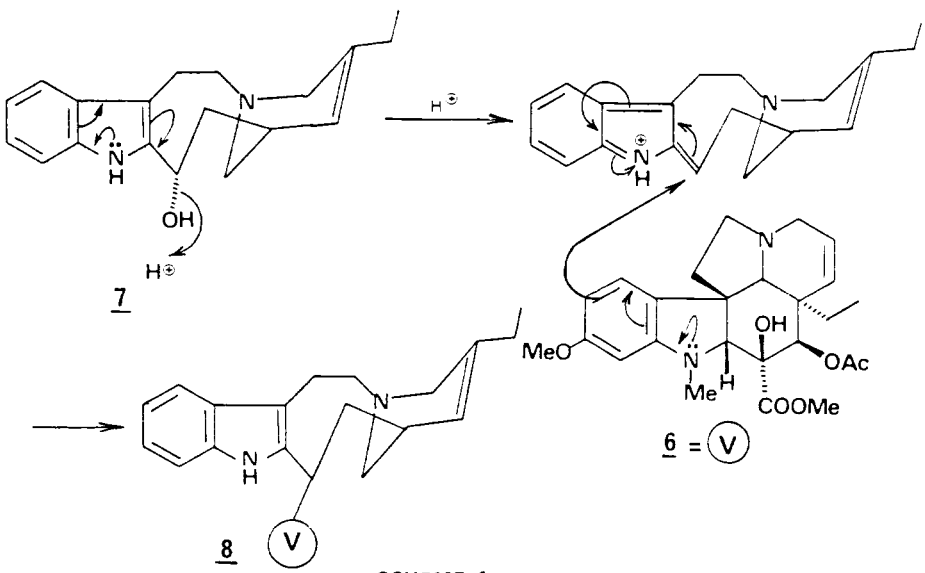


5: CATHARANTHINE



6: VINDOLINE = (V)

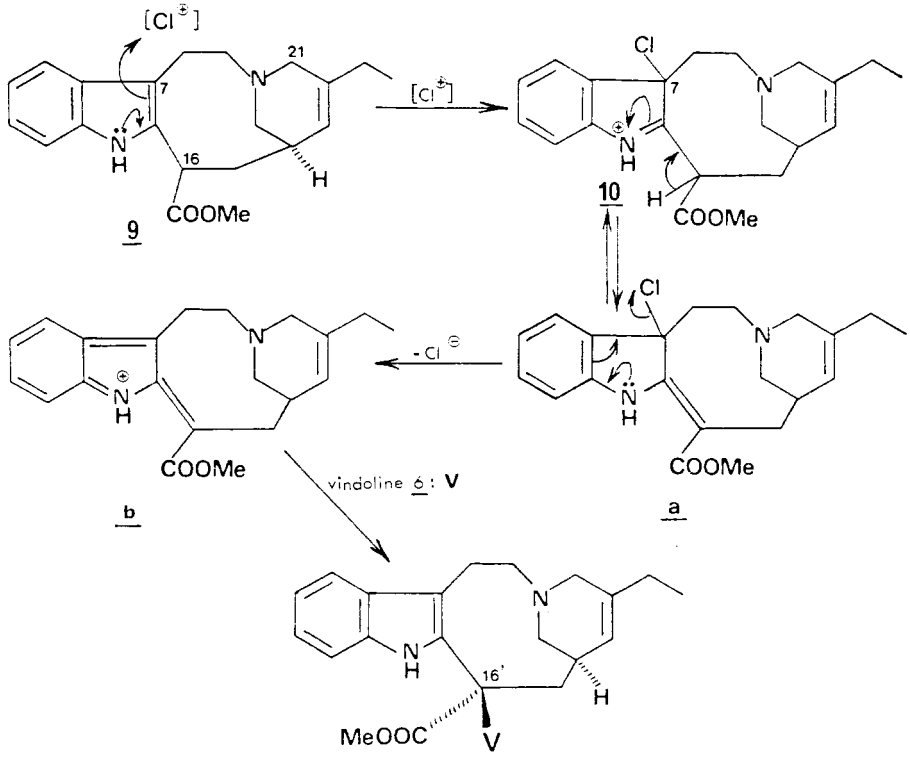
An obvious strategy for achieving the synthesis of these dimeric compounds was, therefore, to prepare the C_{16}-C_{21} secocatharanthine **9**, to activate its C_{16} position, and to allow it to react with vindoline **6**.



SCHEME 1

The *first approach* was devised by Büchi and coworkers (5) and used later by Harley-Mason and Atta-ur-Rahman (6) and also by Bylsma and Kutney (7): 16-hydroxycleavamine **7** is reacted with vindoline **6** in acidic medium leading to compound **8** (scheme 1).

The *second approach* known as the "chloroindolenine approach" consists of treating a convenient indolic compound (i.e. 16,21-secocatharanthine **9**) with a "positive chlorine" donating agent (t-butylhypochlorite, chlorotriazole, etc.). This reaction leads to a 7-chloroindolenine derivative **10** (8, 9, 10) which can react with vindoline **6** giving rise to 16'R anhydrovinblastine **11** (scheme 2) (13, 22).



SCHEME 2

However, the two preceding methods lead to dimeric compounds which do not possess the "natural" (16'S) configuration of the vinblastine alkaloids. The biological consequence of this difference is that the 16'R compounds are devoid of any significant antitumor activity.

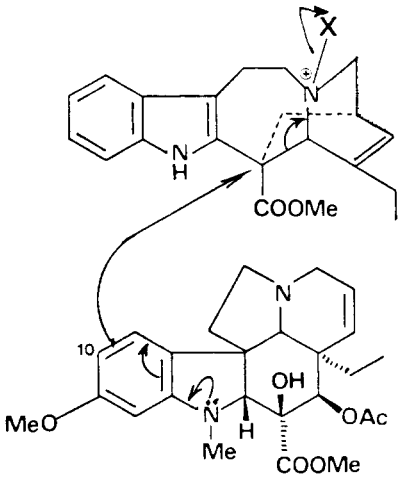
The development of a new method to obviate the configurational problem was, therefore, a worthwhile objective.

We envisaged that the modified Polonovski reaction, which we had discovered in 1967 (11, 12), could provide the stereochemical key to solve this challenge.

First of all, we carefully examined the literature concerning the numerous (ca. 80) alkaloids isolated by previous workers and especially by the Eli Lilly Group (1) from various *Catharanthus* species. It was of interest to note that

none of these alkaloids had a structure directly formed by rupture of the C_{16}-C_{21} bond of catharanthine **5**; in addition, no derivatives of catharanthine functionalized at C_{15} and/or C_{20} were isolated.

These two observations led to the assumption that the dimeric indole alkaloids of the vinblastine group (for example **1**, **2**, **3** or **4**) could well be formed in nature through a fragmentation process initiated by a convenient leaving group on N_b of catharanthine **5** resulting in the rupture of the C_{16}-C_{21} bond of this precursor. Subsequent nucleophilic attack of vindoline **6** (at C_{10}) on the electrophilic C_{16} of catharanthine with the assistance of the indole ring completes the sequence (scheme 3).



SCHEME 3

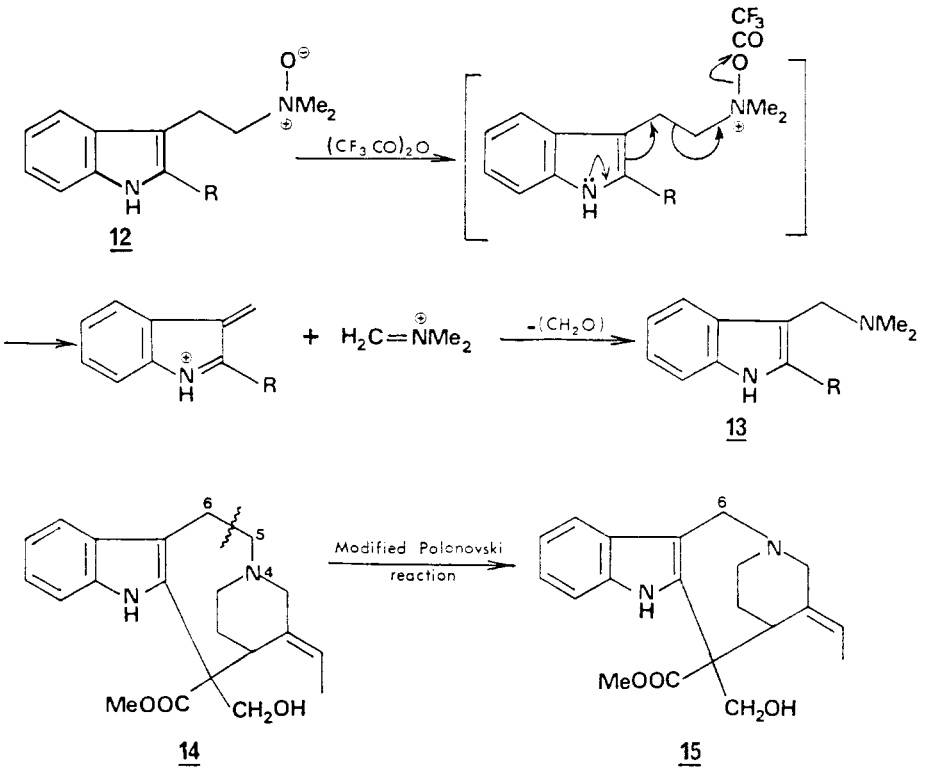
We have already solved a number of synthetic problems, particularly in the indole field, by application of the modified Polonovski reaction. Thus, fragmentation of *N*,*N*-dimethyltryptamine oxide **12** led to a gramine derivative **13** (23). This typical reaction has proved to be very a fruitful one as it was later applied by us and others to achieve some biogenetic-type syntheses: vallesamine **15** from stemmadenine **14** (24) (scheme 4).

The same type of reaction was also applied to the straightforward transformation of the alkaloids of the vobasine-type **16** into those of the ervatamine-type **17** (25):

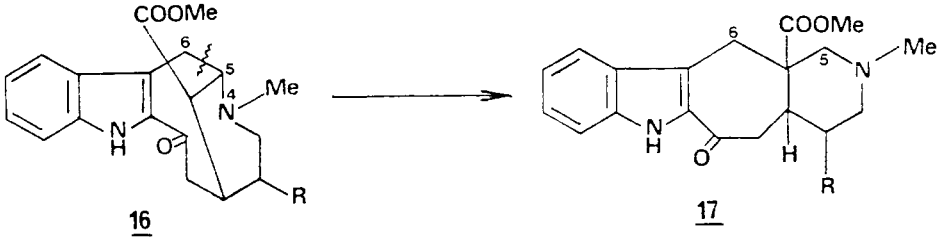
Other applications of the modified Polonovski reaction have been reviewed (26, 27).

In all preceding examples, application of the modified Polonovski reaction to indolic compounds induces a C_5-C_6 fragmentation reaction of the skeleton (**14**→**15**; **16**→**17**).

In the case of catharanthine **5**, however, the situation is expected to be different; indeed, in the catharanthine N_b-oxide **18** the $\equiv N^+-O^-$ bond is antiplanar to the C_{16}-C_{21} bond. Furthermore, the C_{21}-N_b imminium ion **20** to be formed during the fragmentation process will be conjugated to the Δ_{15} (20) double bond (scheme 5). This combination of both steric and electronic factors will then favor the fragmentation of the C_{16}-C_{21} bond over C_5-C_6 fragmentation which has been "normally" observed in the field of tryptamine derivatives.

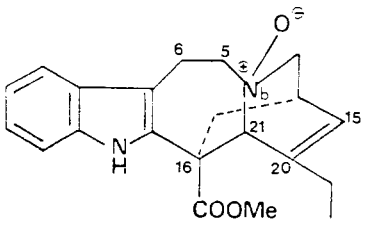


SCHEME 4



This expectation was proved to be correct as, for the first time, 16'S anhydrovinblastine **21** could be obtained as the major product of the modified Polonovski reaction between catharanthine N-oxide **18** and vindoline **6** followed by reduction (28). However, depending on the experimental conditions, a certain amount of the "unnatural" isomer (16'R) **11** was also obtained and its proportion augments as a function of temperature: at −70°C, **21** is practically the only dimeric product to be formed; at −50°C the ratio of *21:11* is about *5:1;* at +20°C, **11** is the unique product formed (4).

This result can be, at least partly, reconciled with conformational equilibria between ions **20** and **22** (27).



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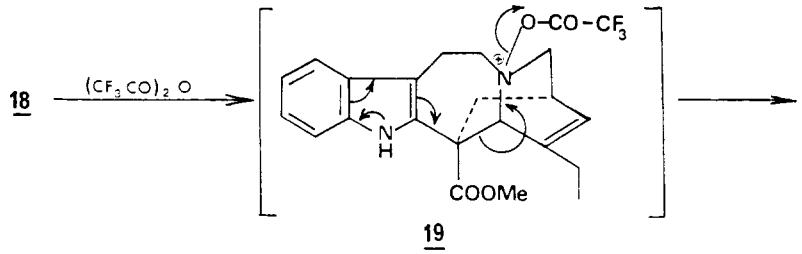

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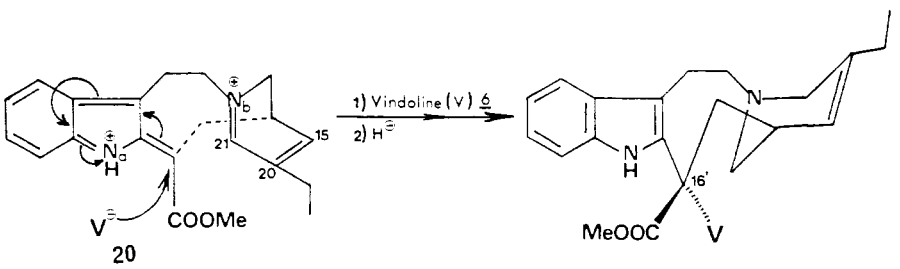

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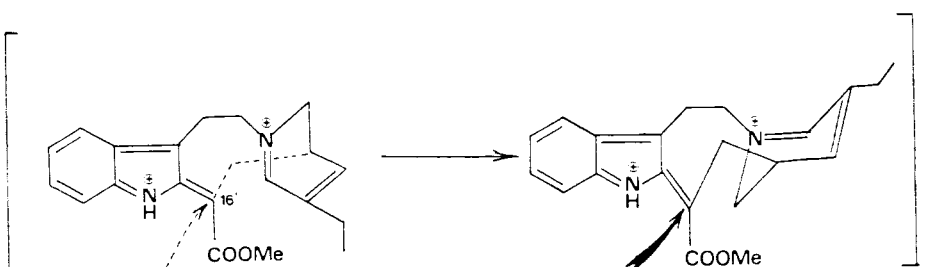
21 : ANHYDRO VINBLASTINE (16'S)
= 16'-EPI 11 (16'R)

SCHEME 5



20 ⟶ 16'S

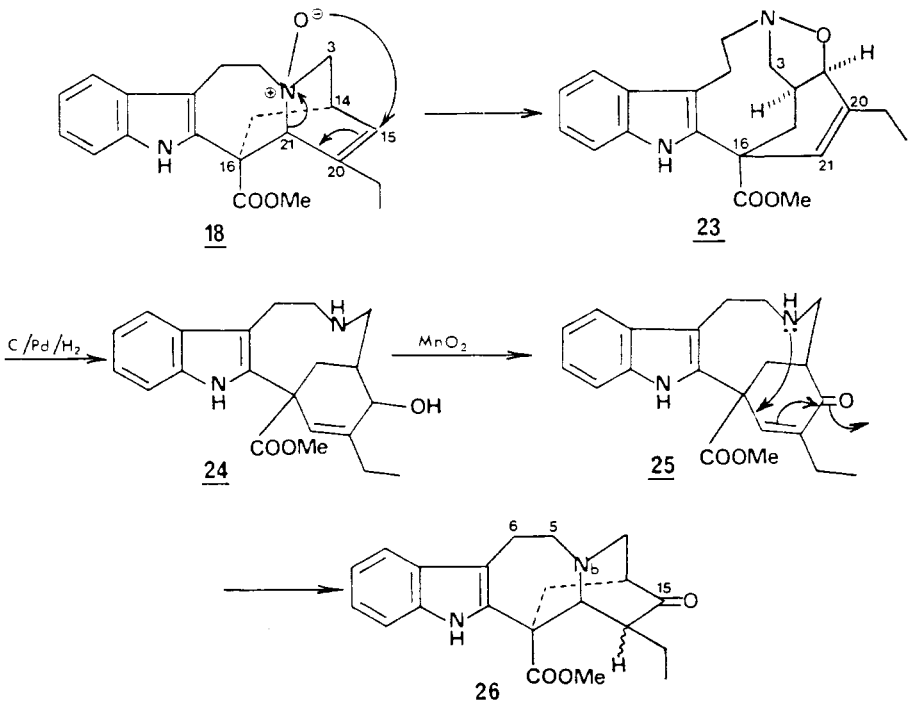
22 ⟶ 16'R

The ion **20** would be the "kinetic" product formed by fragmentation of catharanthine N-oxide **18** (scheme 5) at low temperature. This ion would have the "frozen" conformation of catharanthine which could equilibrate into the "thermodynamic" ion **22** on warming. Inspection of molecular models reveals that nucleophilic attack of **20** by vindoline **6** (v) would lead to compounds of the "natural" series (16'S), while nucleophilic attack by the same vindoline **6** of **22** would lead to the "unnatural" series (16'R). In fact this is by no means the only mechanistic explanation, and other factors such as the formation of charge transfer complexes between reactants have also to be taken into account.

As soon as this important breakthrough in the field of the synthesis of vinblastine-type compounds was known, several other teams used our process of coupling (30, 31, 32, 33). However, coupling of functionalized catharanthine derivatives via their N_b-oxides (derivatives of **18**) with vindoline **6** using our modified Polonovski reaction conditions led to irreproducible (32) or poor (31) yields of the expected dimeric products. This was mainly due to the fact that C_{15} and/or C_{20} functionalized catharanthine derivatives no longer have the $\Delta^{15(20)}$ double bond present (**5** or **18**) and necessary for the C_{16}-C_{21} fragmentation reaction to take precedence over the "normal" C_5-C_6 fragmentation (see above).

Although we eventually developed a different strategy for the functionalization of the $C_{15'}$-$C_{20'}$ region of the anhydrovinblastine **21** which was performed *after* coupling (29), we also investigated the functionalization of catharanthine at C_{15} and/or $C_{20'}$ *prior* to coupling reaction.

For this purpose, we took advantage of the quantitative 2,3-sigmatropic rearrangement of catharanthine N-oxide **18** into **23** to functionalize C_{15} (34): hydrogenolysis of the resultant isoxazolidine **23** led to the aminoalcohol **24**.

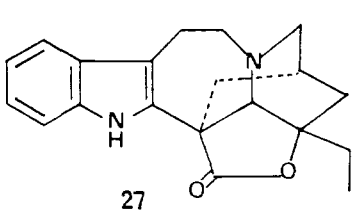


SCHEME 6

Subsequent allylic oxidation gave rise to the conjugated ketone **25**, which underwent an internal Michaël-type addition leading to **26** (scheme 6).

This compound has been coupled with vindoline after tansforming it into its N_b-oxide. Again, one obtains a mixture of products resulting mostly from the C_5-C_6 fragmentation process.

Also, catharanthine lactone **27** and 16-descarbomethoxy catharanthine **28** were coupled with vindoline **6** following our modified Polonovski procedure (35, 36). The yields obtained in the corresponding 16'S dimeric compound were quite poor; as expected, the major dimeric products obtained resulted again from a C_5-C_6 fragmentation reaction of the catharanthine moiety.



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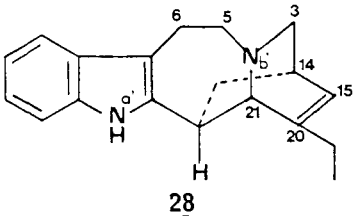

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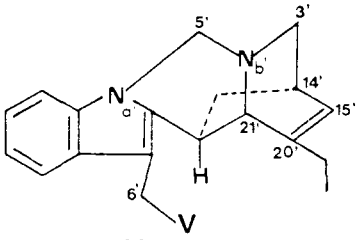

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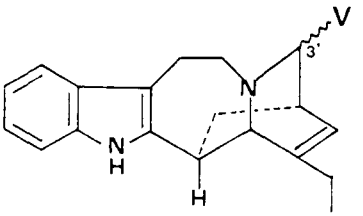


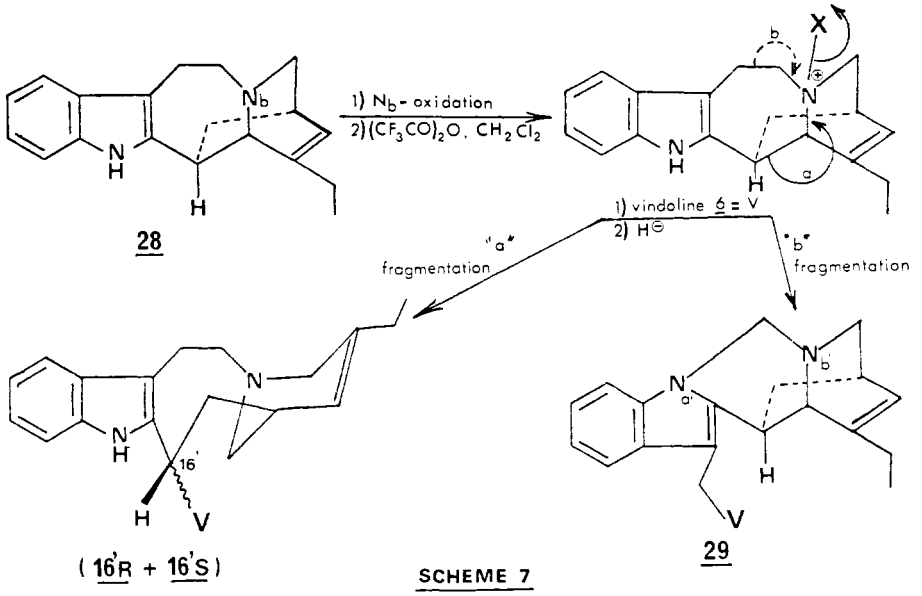
30

For example treatment of 16-descarbomethoxy catharanthine **28** under the modified Polonovski reaction conditions led, in addition to the 16'S and 16'R dimers of the anhydrovinblastine series (C_{16}-C_{21} fragmentation), to a compound **29** (36) resulting from a C_5-C_6 fragmentation process to which formula **30** was wrongly attributed by Kutney and coworkers (33) (scheme 27).

After appraisal of the above results, we concluded that, indeed, our strategical approach for the synthesis of vinblastine-type alkaloids could well be better (29). We therefore investigated this second approach which consists of: first, coupling catharanthine N_b-oxide **18** with vindoline **6** leading to anhydrovinblastine (16'S) **21** and, then, functionalization of the $C_{15'}$-$C_{20'}$ region of this compound. In fact, inspection of the formula **21**, (see also formula **1**, $\Delta^{15'(20')}$), reveals that, in addition to the presence of the $\Delta^{15'(20')}$ double bond of the "upper" catharanthine-derived moiety, account must also be taken of the double bond of the "lower" vindoline moiety. *A priori*, this latter double bond is expected to be even more reactive than the former one ($\Delta^{15'(20')}$). However a molecular model shows that this Δ^{14} double bond is in fact protected by the upper part of the anhydrovinblastine molecule **21**. This conclusion is also borne out by experiment. For example, catalytic hydrogenation of **21** leads quantitatively to the dihydro-15', 20' (S) compound (or deoxy-vinblastine B) (28, 37).

Coming back to the biogenetic aspect of these reactions, we can admit that, in nature, vinblastine-type compounds could well be formed, as already indicated,

from catharanthine **5** and vindoline **6** leading to anhydrovinblastine **21** followed by appropriate functionalization of the $\Delta^{15'(20')}$ region. In addition to our own work, Kutney and coworkers used the same approach after the unsuccessful results obtained in trying to couple, using our process, vindoline **6** with various functionalized catharanthine derivatives. These authors obtained leurosine **2** by treatment of anhydrovinblastine **21** with t-butylhydroperoxide. The configuration of the $C_{15'}$-$C_{20'}$ epoxide was, however, given ambiguously as the reverse of the actual configuration **2** (38).

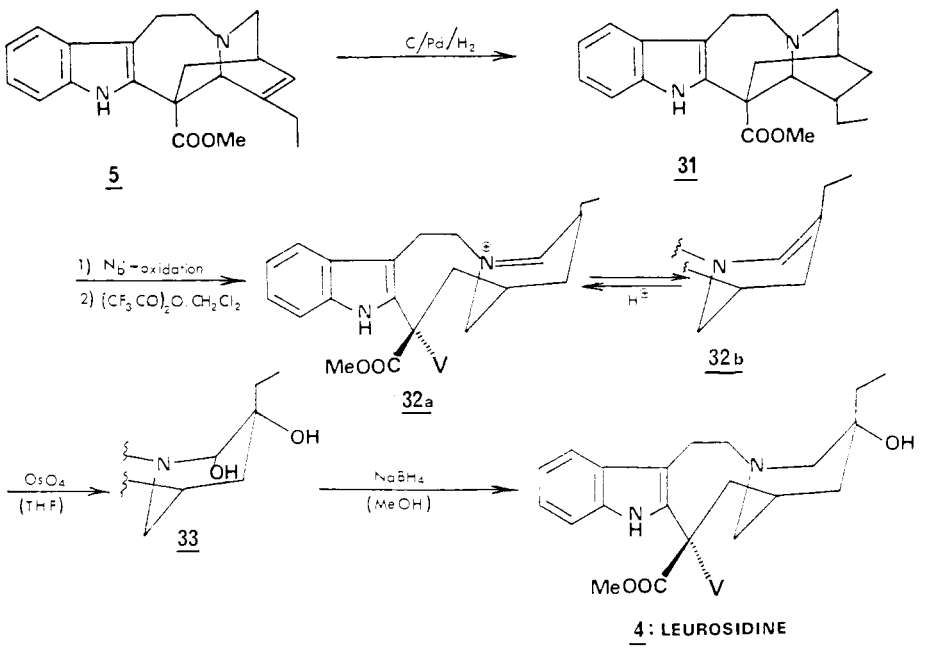


SCHEME 7

If the anhydrovinblastine **21** is well formed in nature, it is particularly striking that this important biogenetic intermediate was never isolated from the various *Catharanthus* species which have been studied so far. The reason for that was evident when we discover the very facile transformation (exposure to air oxygen) of the anhydrovinblastine **21** into leurosine **2** (40–50% yield). This raises the question of whether leurosine **2** is partly or totally an artefact (39). We will return later to this important question.

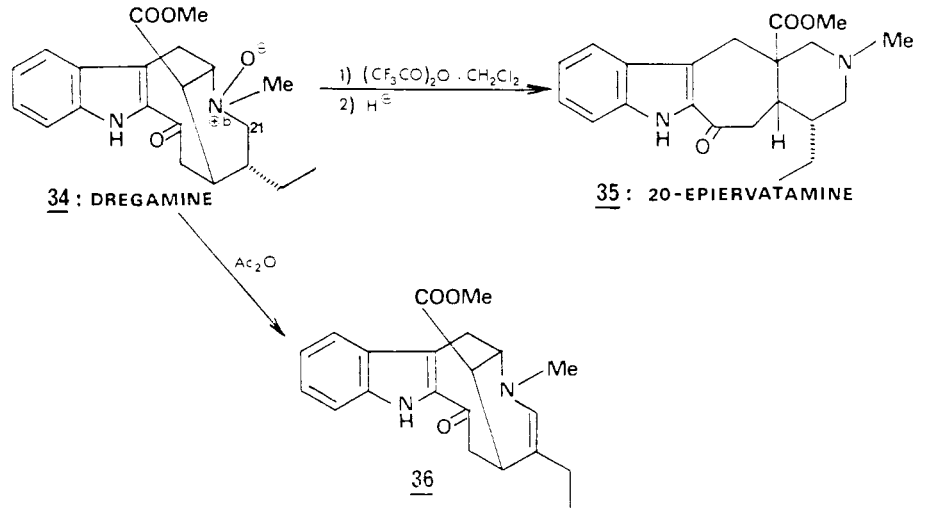
Among the different natural dimeric indole alkaloids of the vinblastine group, some are used in therapeutics: vinblastine **1** and vincristine **3**. Others are known to be active: leurosine **2**, leurosidine **4**. We, therefore, turned our efforts towards the synthesis (with significant yields) of leurosidine **4** and vinblastine **1** itself. It has been shown that vincristine **3** can be easily obtained by oxidation of vinblastine **1** (40).

We first prepared leurosidine **4** (20'-epi vinblastine), after reasoning that an useful intermediate for achieving this goal (as well as for the synthesis of vinblastine **1** itself) would be the enamine **32b**. This enamine was prepared starting from the catalytic hydrogenation product **31** obtained quantitatively from catharanthine **5** followed by coupling of the corresponding N-oxide with vindoline **6**. The desired enamine was treated with OsO_4 in tetrahydrofuran leading to **33**; reduction of this latter carbinolamine with $NaBH_4$ in methanol afforded leurosidine **4** in only 6% yield (41) (scheme 8).



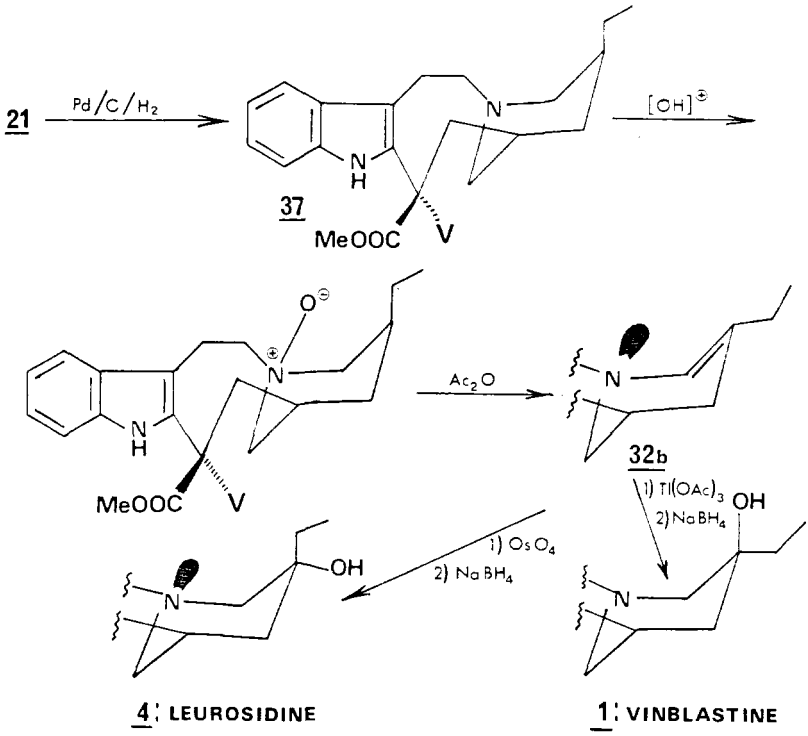
SCHEME 8

A more efficient preparation of both leurosidine **4** and vinblastine itself **1** was found by application of an observation that we made some time earlier (42): treatment of dregamine N_b-oxide **34** with trifluoroacetic anhydride, followed by hydride reduction, led to an almost quantitative yield of the rearranged product **35** of the ervatamine series, via a fragmentation process. However, when **34** is treated with *acetic anhydride*, the major product formed is the enamine **36** resulting from an elimination reaction of one of the two protons in C_{21} (scheme 9).



SCHEME 9

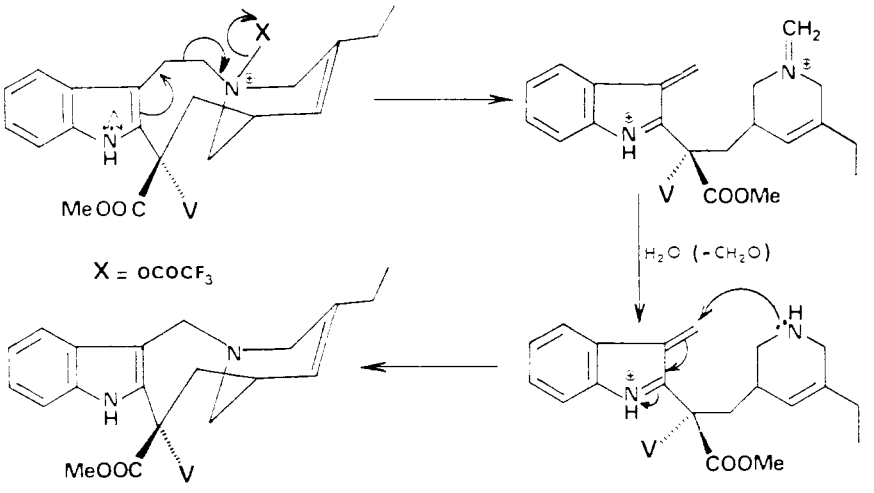
All things being equal, the action of trifluoroacetic anhydride on an N-oxide favors fragmentation, whereas acetic anhydride favors elimination. Therefore, we used this observation for the preparation of the enamine **32b** starting from the N_b-oxide of **37** (obtained by catalytic hydrogenation of anhydrovinblastine **21**). Room temperature reaction of this N-oxide with acetic anhydride followed by distillation *in vacuo* of the excess of reagent led to the (non isolated) enamine **32b**. This enamine **32b** treated with OsO_4 in THF led, after hydride reduction, to leurosidine **4** (30%). The same enamine **32b** treated with thallium triacetate (CH_2Cl_2) followed by sodium borohydride reduction (MeOH) led, after purification, to vinblastine **1** (30%) (43) (scheme 10).



SCHEME 10

The contrasting behavior of the two oxidants towards the enamine **32b** can be rationalized on the following basis. Preferential α attack of the bulky osmium tetroxide reagent from the less hindered face leads, after borohydride reduction of the intermediate carbinolamine **33**, to leurosidine **4**. With thallium triacetate, however, the typical axial β attack on enamine **32b** is observed, leading after borohydride reduction to vinblastine **1**.

NOR-5' VINBLASTINE SERIES: We have previously described the conversion of stemmadenine **14** into vallesamine **15** under the experimental conditions of the modified Polonovski reaction. In an analogous manner, anhydrovinblastine **21** N_b-oxide, without any further reduction, but on treatment with water, led to nor-5' anhydrovinblastine **38** (scheme 11) among other products.



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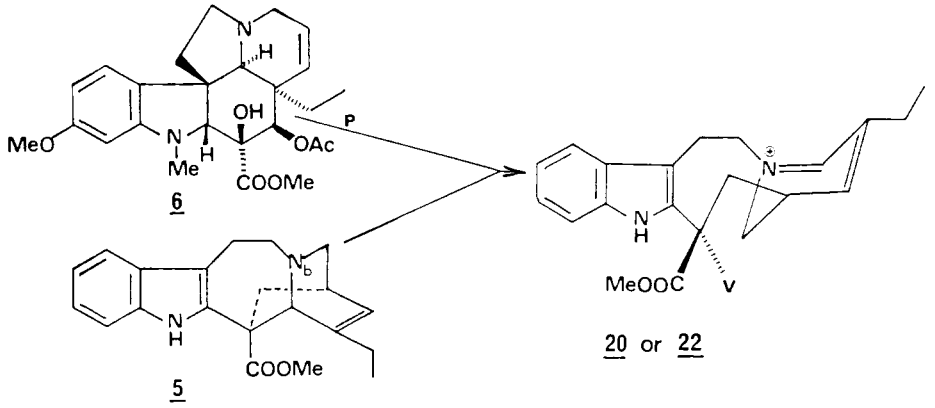
SCHEME 11

This reaction is currently under development in our laboratory and has already enabled us to prepare compounds of the nor-vinblastine series which have revealed interesting antitumor activity.

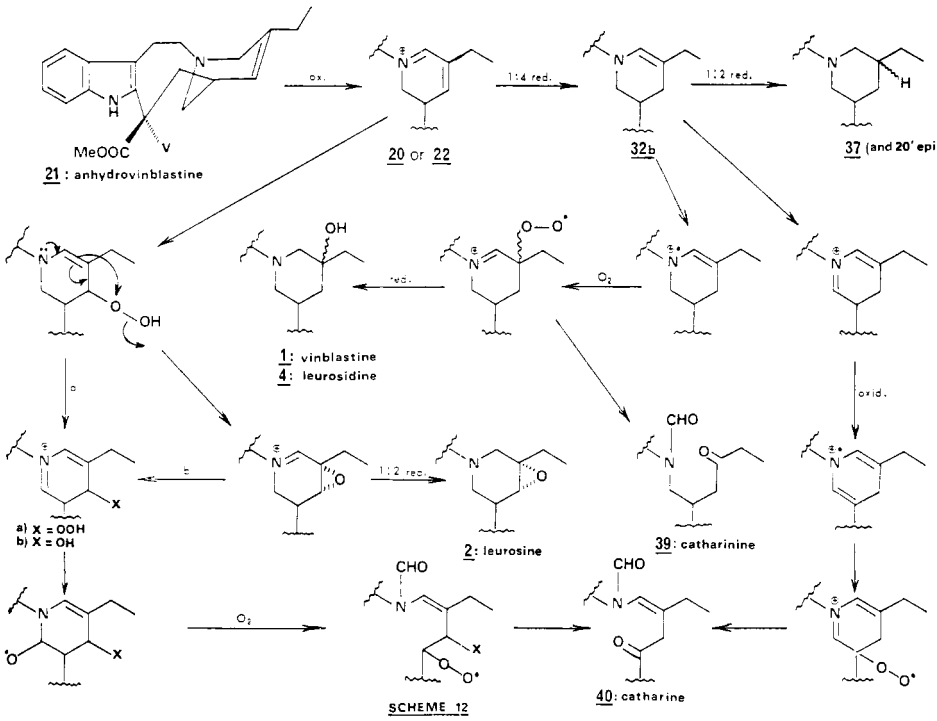
BIOSYNTHESIS OF THE VINBLASTINE-TYPE ALKALOIDS: As soon as the chemical structure of vinblastine was known due to the main efforts of the Eli Lilly Group in Indianapolis, it was clear that this clinically important dimeric indole alkaloid could well be formed in nature from its two obvious precursors present in the same plant: catharanthine **5** and vindoline **6**. This assumption was also made in particular by Atta-ur-Rahman, G. Büchi, C. R. Hutchinson, J. P. Kutney, and A. I. Scott and was used by us for solving the problem of the synthesis of this type of alkaloids.

We have also seen that the first product formed after coupling of vindoline **6** with catharanthine **5** N-oxide (or equivalent) was the ion **20** or **22** which can undergo either 1,2 reduction leading to anhydrovinblastine **21** or 1,4 reduction leading to the enamine **32b** or a number of other oxidation-reduction reactions (see later).



Scott, Guéritte and Lee (17), Hassam and Hutchinson (44), Zenk, Treimer and Stöckigt (45, 46) and recently Stuart, Kutney, Worth and coworkers (47) have contributed to this research. They have demonstrated that anhydrovinblastine **21** was indeed a precursor of leurosine **2** and of vinblastine **1** and other "downstream" oxidation products of **21** (39). Guéritte, Scott and Lee were able to isolate anhydrovinblastine **21** itself (17) from *C. roseus* plants.

However, all the above results have to be interpreted with great caution since we have demonstrated that a number of, if not all, the products biosynthetically derived from anhydrovinblastine **21** can be also formed when an acetonitrile solution of **21** is left to undergo a spontaneous air-oxidation. Furthermore, the relative proportions of the products formed, leurosine **2**, catharine **40**, catharinine **39**, vinblastine **1**, leurosidine **4**, deoxyvinblastine **37** and its 20' epimer, are approximately the same as those in which they are isolated from natural sources (scheme 12). This raises once more the question of the "natural artefacts" (39).



SCHEME 12

BIOLOGICAL ACTIVITY OF VINBLASTINE-TYPE COMPOUNDS: In 1970, we started our research in the field of antitumor alkaloids of the vinblastine group as two of these compounds, vinblastine **1** and vincristine **3**, were constantly used in the treatment of leukemias and related diseases and also of some solid tumors. Some derivatives of these compounds were prepared (N_a-formyl leurosine, vindesine®, etc.). However, all these products were prepared from material isolated from natural sources.

Our success in the synthesis of vinblastine-type alkaloids allowed us (and others) to prepare entirely new derivatives. We made use of the interaction between these compounds (known as "spindle poisons"), and tubulin for selecting

active compounds (49). There is a very good correlation between the response to this *in vitro* test and the antitumor activity of a given compound of the vinblastine series.

We are currently developing new compounds which have exhibited a strong interaction with tubulin in the above test and have been found to be very active in the L 1210 and P. 388 experimental leukemias.

ACKNOWLEDGMENTS

The above work has been realized by Dr. Nicole Langlois, who first prepared the anhydrovinblastine and other "downstream" products with the help of Dr. R. Z. Andriamialisoa. Dr. Y. Langlois was helped by Dr. (Miss) F. Guéritte in developing synthesis of various vinblastine-type alkaloids. Dr. (Miss) Guéritte spent one year at Professor A. I. Scott's laboratory and took part in the important biosynthetic experiments achieved in this laboratory. Dr. P. Mangeney prepared vinblastine and various derivatives in the nor 5'-vinblastine series. The biological testing on tubulin was done by Dr. D. Guénard with the help of Miss F. Zavala. These coworkers are all achnowledged for their skill and their enthusiastic participation in this work.

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ERRATUM

David D. Biesboer and Paul G. Mahlberg: The Effect of Medium Modification and Selected Precursors on Sterol Production by Short-Term Callus Cultures of *Euphorbia tirucalli*. Vol. **42**, No. 6, p. 654.

Figure 4 should have been presented as below:

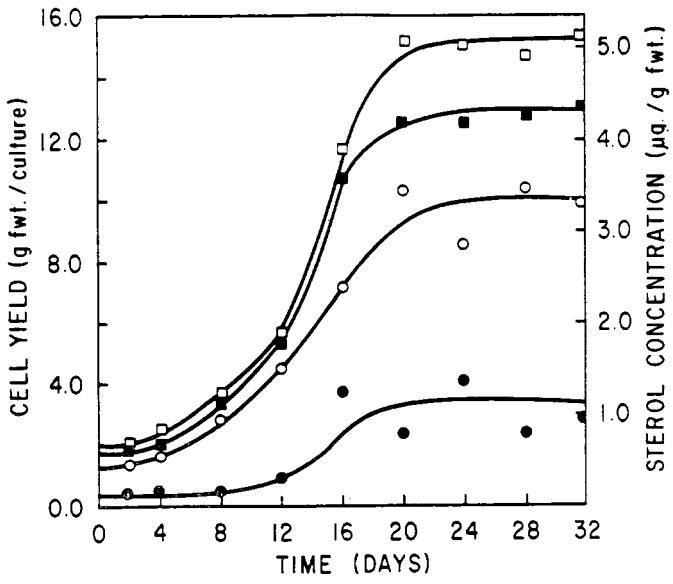

FIG. 4. Kinetics of growth and sterol production by *E. tirucalli* callus cultures. (■)—cell yield; (□)—total sterol yield; (○)—tirucallol; (●)—euphol. Note the variation in ratio of tirucallol/euphol.