No general relationship between the chemistry of colorants and stability has been found. However, the indigoid, pyrazolone, and quinoline types rank high in stability. Xanthene, fluoran, and triphenylmethane dyes rank low. Monoazo and anthraquinone colorants generally are intermediate. Functional groups and their positions no doubt are important factors in colorant stability.

Tablets of R3 were run in duplicate in order to obtain an estimate of reproduciblity. The data for the various color differences and two concentrations of the colorant are summarized in Table II. Color differences for each concentration are of different magnitudes as was noted previously. However, an inverse correlation between color difference and lightness, Y, is seen. For the lower concentration, tablet *1* has a lightness of 73.2 *versus* 74.4 for tablet *2*. Tablet *2* shows a smaller degree of fading in all instances, an effect related to the change in concentration. Even though the tablets contained the same amount of colorant, the compression of the powder gave tablets of variable lightness, a factor that alters stability to some extent. The same correlation can be seen for the higher concentration where tablet *1* is lighter than tablet *2*.

SUMMARY AND CONCLUSIONS

A method of testing color stability using a colorimetric procedure has been described. The same methodology is satisfactory for testing a variety of pharmaceutical products. A ranking of colorant stabilities in tablets is given which is intended to provide a guide for selecting a stable color for related formulations.

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Tissue Culture of *Digitalis mertonensis* I

Effect of Certain Steroids on the Callus Growth and Formation of Baljet Positive Substances in *D. mertonensis*

By R. S. MEDORA*, D. P. N. TSAO, and L. S. ALBERT

Static culture conditions for the growth of *Digitalis mertonensis* callus tissue are described. Effects of different steroidal "precursors" on the growth of this callus tissue and the production of total constituents, positive to the Baljet reagent, in the callus and media are estimated.

THE FIRST work on tissue culture of the genus *Digitalis* was reported by Gautheret (1). Staba and co-workers (2–5) also described the growth and metabolism of static and suspension cultures of *Digitalis purpurea* L., *Digitalis lanata* Ehrh., and *D. purpurea* var. *gloxinaeflora* Hort., including a biotransformation study of digitoxigenin using cultures of *Digitalis mertonensis* Buxton and Darlington.

Steroids have been known to stimulate or inhibit growth in microorganisms and plant tissues (6). Tsao (7) found that 0.25% of sodium glycocholate stimulated and 5% concentrations inhibited glycoside production in *D. purpurea*. On the other hand, Chan and Tsao (8) found that sodium cholate inhibited glycoside

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* Present address: Botany Department, McGill University, Montreal, Quebec, Canada.

production in *D. purpurea* in concentrations of 0.5 and 2.5%. Much (9) has shown that 2% of cholesterol stimulated growth of digitalis while Tsao and Youngken (10) showed that 0.5 and 2% of cholesterol increased the glycosides in the leaves of *D. purpurea*. Buvat (11) and Levine (12) found that the degradation products of cholesterol produced more callus and organ growth in other species of plants.

These and other findings prompted the authors to investigate the effects of certain solubilized sterol mixtures and cholesterol on glycoside production when added to a medium for the callus growth of *D. mertonensis*.

EXPERIMENTAL

Sterol *A*,[1] sterol *B*,[2] and pure cholesterol[3] were selected as steroidal "precursors" to study their effects on the growth and glycoside production in the callus tissue of *D. mertonensis*. Sterol *A* (13,14) is an oil-soluble, hydrophilic, multisterol, nonionic surfactant, emolient, emulsifier, and comprises mainly the sterols from lanolin solubilized in a vehicle. Sterol *B* (13, 14) is a stable, active, water-soluble, ethoxylated derivative of cholesterol. The sterol *B* molecule is comprised of 25% pure cholesterol and 75% of the 24-molecule ethylene oxide side chain.

Preparation of the Media for Static Cultures.—Except where specified, all inorganic compounds used were of analytical reagent grade and the organic compounds[4] employed were of better available grades. A modification of White's (15) basic media was used for all experiments carried out with a semisolid media. In addition to vitamins of Eagle's solution (16), *myo*-inositol, biotin, and nicotinic acid in quantities of 200 mg., 1 mg., and 0.4 mg./L., respectively, were also included in the list of vitamins. Among others were sodium ferric ethylenediaminetetraacetate (NaFeEDTA), glycine, coconut water, sucrose, and the supplement suggested by Staba (2) for growth of digitalis callus tissue. Agar (0.7%) was added to give a semisolid consistency to the media, and 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-benzothiazoleoxyacetic acid (BTOA) in quantities of 1 mg. and 2 mg./L., respectively, were used to induce callus formation. The ingredients in formula 1 (Table I) were dissolved in glass distilled water and if necessary adjusted between pH 5.4–5.7 with potassium hydroxide. The media was then distributed into 125-ml. wide mouth conical flasks plugged with nonabsorbent cotten, covered with paper cups, and autoclaved. The ingredients of the supplement [formula 2 (Table I)] also were dissolved in glass distilled water, adjusted to the same pH as above, passed through a Millipore[5] HA filter and aseptically added to the luke warm autoclaved flasks. Totally, 20 ml. of semisolid sterile media (formulas 1 and 2) was then used in each flask to grow callus tissue.

TABLE I.—MEDIA FOR STATIC CULTURE

	mg./L.
Formula 1	
White's Modified Basal Media	
$MgSO_4 \cdot 7H_2O$	625
$Ca(NO_3)_2 \cdot 4H_2O$	200
Na_2SO_4 (anhydrous)	200
KNO_3	80
KCl	65
$NaH_2PO_4 \cdot H_2O$	19
$MnSO_4 \cdot H_2O$	4.5
$ZnSO_4 \cdot 7H_2O$	2.6
H_3BO_3	1.3
KI	0.75
$Na_2MoO_4 \cdot 2H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.003
Other Ingredients Used	
Sucrose	20,000
Agar[a]	7,000
Sodium ferric ethylenediaminetetraacetate[b]	50
Glycine	2
2,4-Dichlorophenoxyacetic acid	1
2-Benzothiazoleoxyacetic acid[c]	2
Coconut water	150 ml./L.
Eagle's Modified Formula for Vitamins (16)	
Formula 2	
Staba's Supplement (2)	

[a] Difco Laboratories, Detroit, Mich. [b] Geigy Industrial Chemicals, Ardsley, N. Y. [c] American Cyanamid Co., Princeton, N. J.

Conditions of Growth and Incubation.—MacLachlan (17) has noted that soybean seedling produced more sterols in the dark than in the light. Blackley and Stewart (18) reported that suspension cultures of *Hoplopappus* grown in the dark are more friable. It was the experience of the authors that static cultures grown in the dark were lighter in color and more friable. Friable cultures have been considered better for initiating suspension cultures (19). As the authors were interested in cultures which produced steroidal glycosides, all experiments were carried out in complete darkness.

All plant tissues were incubated in a Hotpack constant temperature room[6] model 1288-14 (operating temperatures 0 to 40°) adjusted to 27 ± 1°. The humidity of the incubator was maintained 50 to 80% by a De Vilbiss vaporizer[7] model 144.

Aseptic manipulations were carried out between Bunsen burners or in a sterile transfer chamber[8] which maintained positive air pressure and which was equipped with an ultraviolet lamp. The seeds of *D. mertonensis* were obtained from the Drug Plant Gardens, University of Washington, Seattle. They were sterilized by the method described by Staba (2). The sterile seeds were then distributed in Petri dishes, 9 cm. in diameter, which contained disks of Whatman No. 1 filter paper soaked in 10

[1] Marketed as Amerchol L-101 by American Cholesterol Products, Inc., Edison, N. J.
[2] Marketed as Solulan C-24 by American Cholesterol Products, Inc., Edison, N. J.
[3] Merck & Co., Inc., Rahway, N. J.
[4] Nutritional Biochemicals Corp., Cleveland, Ohio, and Calbiochem, Los Angeles, Calif.
[5] Millipore Filter Corp., Bedford, Mass.
[6] Hotpack Corp., Philadelphia, Pa.
[7] The De Vilbiss Co., Somerset, Pa.
[8] Forma Scientific, Inc., Marietta, Ohio.

to 12 ml. of distilled water. The seeds were then incubated for 14 days. On the 14th day two germinated seedlings were aseptically transferred to each conical flask containing sterile media for static tissue culture. The seedlings were allowed to grow and develop into callus tissue. On the 37th day in all experiments the callus tissue from the first generation of growth was harvested, weighed (fresh weight), dried at 50° for 72 hr., and weighed again (dry weight).

Extraction of Callus from Static Culture.—The dried callus was weighed and coarsely ground. A tincture was prepared according to the U.S.P. method for digitalis tincture (20). The callus tincture was then stored in a refrigerator until used.

Extraction of the Culture Media.—On the basis of the moisture present in the media, enough alcohol was added to the media to give the same alcohol concentration as that of the menstruum used to extract the callus. Later, more menstruum was added, so that the total volume of menstruum corresponded to 2 ml. for each milliliter of the media at the start of incubation. The flasks with the media and menstruum were then shaken for 24 hr. The extractive from each lot was pooled separately, filtered through cheese cloth, and filtered twice through Whatman No. 1 filter paper. The clear extractive was evaporated to a syrupy consistency in a flash evaporator at temperatures below 50° and then lyophilized. The lyophilized extract was weighed and stored in a desiccator for future use. Two grams of the lyophilized extract was shaken for 8 hr. in 10 ml. of the menstruum. The solution was then filtered and the tincture stored for future use.

Colorimetric Assay of Baljet Positive Constituents[9] in the Callus and Media.—The assay method of the standard preparation of digitoxin in the U.S.P. XVII (21) was closely followed and a standard curve was plotted with micrograms of digitoxin *versus* absorbance. The total amounts of constituents in the callus and media tinctures were calculated as digitoxin and were determined by the modified method of Knudson and Dresbach (22) after preliminary decolorization of the tincture. To a suitable aliquot of decolorized tincture an equal volume of alkaline picrate was added. The absorbance of this solution was read on a Beckman DU spectrophotometer[10] at 495 mμ 16 min. after the addition of the alkaline picrate, against a blank using 0.5-cm. cells. The blank was prepared exactly the same way as the tincture, using aliquots of menstruum instead of the tincture.

Experiment A.—In the preliminary experiments, the concentration of 0.5% of sterol *A* was found to be optimum for the growth of the callus tissue on the basis of its dry weight. Therefore, 0.5% of sterol *A* was added to the media of some lots in this experiment. Equal quantities of the media for the growth of callus tissue were distributed in flasks with the Brewer automatic pipeting machine,[11] model 07-502, The flasks were divided into 6 lots. To the media of lots 1 and 4, 0.5% of sterol *A* was added while an equivalent amount of vehicle[12] was incorporated in lots 2 and 5. Lots 3 and 6 were used as controls. The media was prepared exactly as described earlier, except that sterol *A* was incorporated in formula 1 for lots 1 and 4 while the vehicle of sterol *A* was added to formula 1 for lots 2 and 5. Therefore, both sterol *A* and its vehicle were subjected to autoclaving with the rest of the media before ingredients of formula 2 were aseptically added. In all experiments, the callus tissue was grown in flasks of lots 1, 2, and 3, while lots 4, 5, and 6, respectively, were kept as controls for the above lots. For all experiments, the callus from each lot was harvested on the 37th day, dried, and the total constituents determined colorimetrically (Table II). The media tincture was also analyzed for the presence of total constituents (Table III).

Experiment B.—The lanolin fraction containing sterols in sterol *A* is about 1.2% (23). Sterol *B* is a molecule containing 25% by weight of pure cholesterol and 75% by weight of 24 moles of ethylene oxide side chain (14). On the basis of these data, enough sterol *B* was added to the nutrient media, such that the weight of cholesterol in it was approximately equivalent in weight to the total sterols present in sterol *A* previously added.

The side chain of sterol *B* is made up of 24 molecules of ethylene oxide and this is approximately equivalent to a molecule of polyethylene glycol 1000.[13] Therefore, polyethylene glycol 1000 was used in the media of one lot along with sterol *B* in another. Polyethylene glycol 1000 was added to the media of lots 2 and 5 in quantities equivalent to 75% of the sterol *B* added above. These lots were especially prepared to determine whether the 24 molecule ethylene oxide side chain of sterol *B* had an independent effect of its own on the callus tissue.

Totally, 6 lots were prepared for this experiment. Lots 1 and 4 contained 0.024% of sterol *B* in the media, lots 2 and 5 contained 0.018% polyethylene glycol 1000 and lots 3 and 6 were used as controls. The media was prepared the same way as described earlier. Sterol *B* and polyethylene glycol 1000 were added as extra ingredients to formula 1 of the media. The callus from each lot was harvested separately and the constituents were determined in the callus and media (Tables II and III).

Experiment C.—This experiment was carried out to determine whether pure cholesterol had any effect on the callus tissue. In the preliminary experiments, different methods were tried to incorporate cholesterol in the nutrient media in a fine suspension and the method which gave the finest suspension was employed. The ingredients of formula 1 were mixed together as described earlier and then cholesterol dissolved in chloroform was poured in the warm media agitating its container continuously. This gave the finest suspension of cholesterol. The flasks were then sterilized and the ingredients of formula 2 were added after filtration through a Millipore filter.

For this experiment 6 lots were prepared. To the flasks in lots 1 and 4 totally 0.1-ml. quantities

[9] Baljet positive constituents will be referred to as merely "constituents." Throughout the paper, constituents will be defined as total extractives of callus tissue and culture media which give a positive reaction to alkaline picric acid.

[10] Beckman Instruments, Inc., Fullerton, Calif.

[11] Baltimore Biological Laboratory Division, Becton, Dickinson and Co., Baltimore, Md.

[12] The vehicle was supplied by the American Cholesterol Co. and was devoid of the lanolin fraction.

[13] Marketed as Carbowax 1000 by Union Carbide Corp., New York, N. Y.

TABLE II.—ANALYSIS OF THE BALJET POSITIVE CONSTITUENTS[a] OF *D. mertonensis* CALLUS

Sample[b]	Treatment	Seedlings,[c] No.	Fresh Wt., mg.	Dry Wt. mg.	Dry Wt. % Difference	Baljet Positive Constituents mcg.	Baljet Positive Constituents Per mg. Dry Wt. mcg.	Baljet Positive Constituents Per mg. Dry Wt. % Difference
A1c	Sterol *A*	60	20110	1560	+13.87 *vs.* A3c +9.86 *vs.* A2c	2142	1.373	+31.76 *vs.* A3c +13.84 *vs.* A2c
A2c	Vehicle	60	18420	1420	+3.65 *vs.* A3c	1713	1.206	+15.73 *vs.* A3c
A3c	Nil	60	16560	1370		1428	1.042	
B1c	Sterol *B*	68	16750	1290	−20.86 *vs.* B3c	1713	1.327	+1.06 *vs.* B3c −5.75 *vs.* B2c
B2c	Polyethylene glycol 1000	68	24360	1520	−6.75 *vs.* B3c	2142	1.409	+7.23 *vs.* B3c
B3c	Nil	68	25710	1630		2142	1.314	
C1c	Cholesterol	38	7140	790	+21.54 *vs.* C3c +6.75 *vs.* C2c	999.6	1.265	+4.7 *vs.* C3c −6.4 *vs.* C2c
C2c	Chloroform	38	6830	740	+13.85 *vs.* C3c	999.6	1.351	+11 *vs.* C3c
C3c	Nil	38	4160	650		785.4	1.208	

[a] Calculated as digitoxin on the basis of the digitoxin standard curve. [b] The capital letters A, B, and C signify the experiment. The arabic numbers indicate treatment of the media, and "c" stands for callus extracts. Number 3 in all experiments is the control and indicates no treatment. [c] All seedlings were incubated for 37 days in the sterile nutrient media after preliminary germination of the seeds in Petri dishes for a period of 2 weeks.

TABLE III.—ANALYSIS OF THE BALJET POSITIVE CONSTITUENTS[a] OF THE MEDIA IN WHICH *D. mertonensis* CALLUS IS GROWN

Sample[b]	Treatment	Total Dry Extract from Media, mg.	Baljet Positive Constituents in 2 Gm. Extract mcg.	Baljet Positive Constituents in 2 Gm. Extract Corrected for Controls, mcg.	Baljet Positive Constituents in Total Extract, mcg.	Baljet Positive Constituents/mg. Extract mcg.	Baljet Positive Constituents/mg. Extract % Difference
A1m	Sterol *A*	13270	2523	1952	12951	0.975	−13.0 *vs.* A3m +13.9 *vs.* A2m
A2m	Vehicle	12710	2332	1716	10905	0.857	−23.5 *vs.* A3m
A3m	Nil	12560	2853	2237	14048	1.118	
A4m	Sterol *A*	6260	571				
A5m	Vehicle	6570	616				
A6m	Nil	5990	616				
B1m	Sterol *B*	12550	2616	2046	12838	1.022	+19.23 *vs.* B3m +16.25 *vs.* B2m
B2m	Polyethylene glycol 1000	12050	2283	1760	10604	0.88	+2.56 *vs.* B3m
B3m	Nil	12610	2330	1714	10806	0.856	
B4m	Sterol *B*	4350	570				
B5m	Polyethylene glycol 1000	4030	523				
B6m	Nil	3870	616				
C1m	Cholesterol	6590	1666	1190	3921	0.594	+47 *vs.* C3m +4 *vs.* C2m
C2m	Chloroform	7250	1618.4	1142.4	4141.2	0.571	+41 *vs.* C3m
C3m	Nil	7110	1285.2	809.2	2867.7	0.404	
C4m	Cholesterol	7000	476				
C5m	Chloroform	6860	476				
C6m	Nil	7740	476				

[a] Calculated as digitoxin on the basis of the digitoxin standard curve. [b] The capital alphabets A, B, and C signify the experiments. The arabic numbers indicate treatment of the media, and "m" stands for the media extracts. In lots No. 3 and 6 in each experiment the media was not treated. In all experiments, No. 1, 2, and 3 indicate callus grown lots and No. 4, 5, and 6, respectively, are controls of the above lots with no callus grown in them.

of 20% cholesterol solution in chloroform were added with a microburet. To flasks in lots 2 and 5, which were controls, 0.1 ml. of chloroform was added, and lots 3 and 6 served as controls without any adjuncts. The callus from each lot was harvested and dried. The tinctures of callus and media were assayed for their constituents (Tables II and III).

RESULTS AND DISCUSSION

Some of the seeds grown in distilled water sprouted on the 3rd day of incubation while more than 50% of the total viable seeds sprouted by the end of the first week. The seedlings were allowed to grow in the dark for 2 weeks; at the end of this period the radicle sizes varied from 1–2 in. The seedlings grown in the untreated media had the following pattern of growth. On the 6th day after the seedlings had been transferred to the nutrient media, a thickening was observed at the proximal end of the radicle, while the radicle tips of some seedlings showed slight browning. After the 10th day of in-

cubation, the thickening began to appear all over the radicles and they turned light brown. By the time the seedlings had been incubated for 12 days the thickening was very profuse and turned into a homogeneous mass of cells (callus). Some masses of callus also started to give off branches with fluffy protuberances. From this point on, the callus tissue grew larger in size, in the form of interrupted nodules, and started to turn darker in color. When this callus tissue from the first generation of growth was harvested on the 37th day and examined, it was found to be a friable, undifferentiated mass of homogeneous cells.

The callus grown in the media treated with sterol *A* grew in the form of clumps, was more friable, had more protuberances, and was lighter in color. Callus in the vehicle, cholesterol, chloroform, and polyethylene glycol 1000 treated lots had a more or less similar pattern of growth as the controls. In the case of sterol *B*-treated media, the seedlings turned yellow and then light brown even before callus growth was observed. Growth was poorer than the controls and callus tissue did not appear until the beginning of the 3rd week. The development of callus in the cholesterol-treated media was also delayed by about 2 days as compared with the controls.

In experiment *A*, sterol *A* used in 0.5% quantities in the media, showed an increase in the weights of fresh and dry callus as compared with its control (Table II). The callus from the lot treated with the vehicle showed an increase of 3.65% of the dry weight as compared with its control. However, the fact that the callus from the sterol *A*-treated media was 9.86% higher in weight than the one treated with the vehicle, showed that something else in sterol *A* besides the vehicle stimulated the growth of the callus.

In the colorimetric assay, the tincture prepared from the callus grown in the sterol *B*-treated media (Alc), showed an increase of 13.84% of the constituents per milligram dry weight of the callus compared to tincture of the callus grown in media treated with the vehicle (A2c). Compared to the tincture of the untreated callus (A3c), the increase in constituents in the tincture Alc is almost twice as much as that in A2c. Therefore, it could be surmised that, although the vehicle portion of sterol *A* has a stimulating effect on the production of constituents in the callus, the lanolin portion in sterol *A* also has a stimulating effect of its own.

The relatively high amounts of constituents in the media tinctures (Table III) are difficult to explain, particularly because the separated constituents of the media extract on a thin-layer plate are negative to Kedde's reagent. It seems, therefore, that while trying to extract the steroidal glycosides from the media, some portion of the media ingredients, along with other callus metabolites released in the media, were also extracted. Rowson (24) has pointed out that discrepancies do arise if impurities are present in the tinctures assayed for cardiac glycosides. The tincture of the untreated media (A3m), the tincture of the sterol *A*-treated media (Alm), and that of the vehicle-treated media (A2m) in which callus was grown gave a much higher reading for constituents than the tinctures of their respective controls. This indicates that during its growth the callus may have released certain constituents in the media. These constituents do not seem to resemble the digitalis glycosides as they were negative to Kedde's reagent.

From the evidence available, it is difficult to pinpoint which compounds in addition to digitalis glycosides produced a color with alkaline picrate. However, compounds such as glucose, ascorbic acid, creatinine, and lactams of α-guanidine acids, commonly found as plant and animal metabolites, are known to react with alkaline picrate (25, 26). Ascorbic acid has been known to occur in the genus digitalis (27) and Staba (3) has shown that *D. purpurea* suspension cultures hydrolyze sucrose to glucose and fructose. In spite of this, it is unlikely that the sugars are responsible for the color reaction if the absorbance is noted within 20 min. after the addition of the Baljet reagent (25).

The metabolites which react with alkaline picrate seem to be in higher quantities in tincture of untreated media than the sterol *A*-treated one. The reverse is true in the callus tincture, *i.e.*, there are greater amounts of the constituents in the callus from the treated media than from the untreated one. The A2m tincture seems to have smaller amounts of constituents compared with A3m. It seems possible, therefore, that in the absence of the vehicle or the vehicle with the lanolin fraction, more constituents are leached out of the callus cells into the media.

In experiment *B*, the presence of both sterol *B* and polyethylene glycol 1000 in the media seemed to inhibit growth of the callus tissue (Table II). However, the presence of polyethylene glycol 1000 in the media increased the constituents per milligram dry weight of the callus as compared to its control. On the other hand the presence of sterol *B* in the media did not seem to bring about a significant change in the constituents per miligram dry weight of the callus.

There was a significant increase in constituents in the media containing sterol *B*, although only a slight increase in constituents in the media containing polyethylene glycol 1000. The data obtained (Tables II and III) of the growth, constituents of callus, and release of constituents in the media for sterol *B* and polyethylene glycol 1000 treatment vary considerably. However, it is probable the 24-molecule ethylene oxide side chain on sterol *B* is not so inhibitory to growth of the callus as is the cholesterol moiety itself.

In experiment *C*, due to the breakdown in the refrigeration mechanism of the growth chamber, the temperatures in the chamber rose above 27° on various occasions. This retarded the growth of the tissue and the final yield of callus at harvest was less in weight compared to experiments *A* and *B*. Because of the fluctuation in temperature, the authors are skeptical about the results of experiment *C*.

The dry weight of the callus harvested from the cholesterol-treated media was 21.54% higher than the callus harvested from the untreated media; however, only 6.75% higher than the chloroform-treated media. The chloroform-treated lot showed an increase in 13.85% compared to its control. After autoclaving the media, it seems doubtful that enough chloroform stayed behind to produce an effect on the callus growth. However, if some did stay behind, the stimulating effect of less than

0.5% chloroform on the callus is not surprising in light of the findings that solvents such as alcohol in concentration of 0.2 to 0.3% enhanced Avena coleoptile elongation (28).

There is a decrease in the constituents in the callus harvested from the media treated with cholesterol as compared to the callus obtained from its chloroform control. However, in case of the media tincture, the condition is reversed. Because of fluctuation in temperature during experiment *C*, it is not possible to draw any definite conclusions.

From the over-all data of experiment *A*, it seems reasonable to recommend 0.5% sterol *A* as an extra adjunct to the media for the cultivation of static callus cultures of *D. mertonensis*.

SUMMARY

A method is described for the growth of callus tissue from the seedlings of *D. mertonensis* in tissue culture. Certain sterols were also studied for their effects on callus growth and digitalis-like glycoside production.

Increases in callus growth and constituents positive to the Baljet test were observed when a solubilized lanolin fraction sterol *A* was added to the nutrient media in amounts of 0.5%.

Cholesterol, as an adjunct to the culture media in quantities of 0.1%, stimulated callus growth. However, 0.024% of an ethoxylated derivative of cholesterol sterol *B* with a 24-molecule ethylene oxide side chain and a polymer of ethylene oxide (polyethylene glycol 1000) in quantities of 0.018% inhibited callus growth.

Constituents in the callus, positive to the Baljet test increased slightly in the presence of cholesterol and polyethylene glycol 1000. There was no significant change in the callus constituents when sterol *B* was added to the media.

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Heats of Transition of Methylprednisolone and Sulfathiazole by a Differential Thermal Analysis Method

By J. KEITH GUILLORY

Heats of transition and fusion have been determined for methylprednisolone and sulfathiazole by differential thermal analysis calorimetry. Methylprednisolone undergoes an endothermic transition at 209°, with heat of transition, 1380 ± 50 cal. mole^{-1}. Fusion occurs at 240°, with heat of fusion, 5350 ± 200 cal. mole^{-1}. Sulfathiazole undergoes an endothermic transition at 161°, with heat of transition, 1420 ± 40 cal. mole^{-1}. Fusion occurs at 200°, with heat of fusion, 5960 ±210 cal. mole^{-1}.

As part of a program investigating the phenomenon of polymorphism in pharmaceuticals it was desirable to obtain reliable data on the heats of transition and fusion for a large number of compounds. Adiabatic calorimetry (1), solubility measurements (2–4), and differential thermal analysis were among the techniques considered for use in these determinations. Calorimetry was rejected as being too time consuming for routine analyses. There are a num-

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